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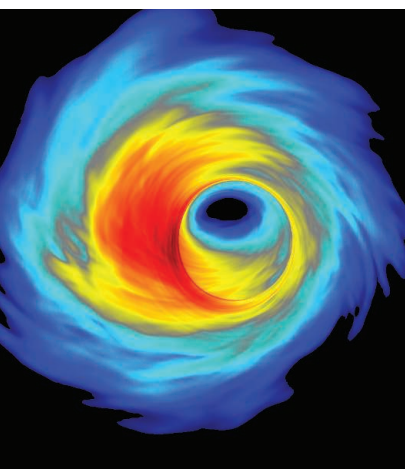
COVER

Young whooping cranes (*Grus americana*) follow an ultralight aircraft. Each year, endangered whooping cranes are reared in captivity and artificially trained by ultralight aircraft on their first lifetime migration from Wisconsin to Florida. Analyses of data from subsequent unaided migration events show that migratory performance improves over many years and that social learning from older birds plays an important role. See page 999.

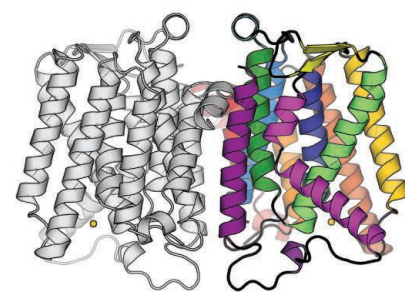
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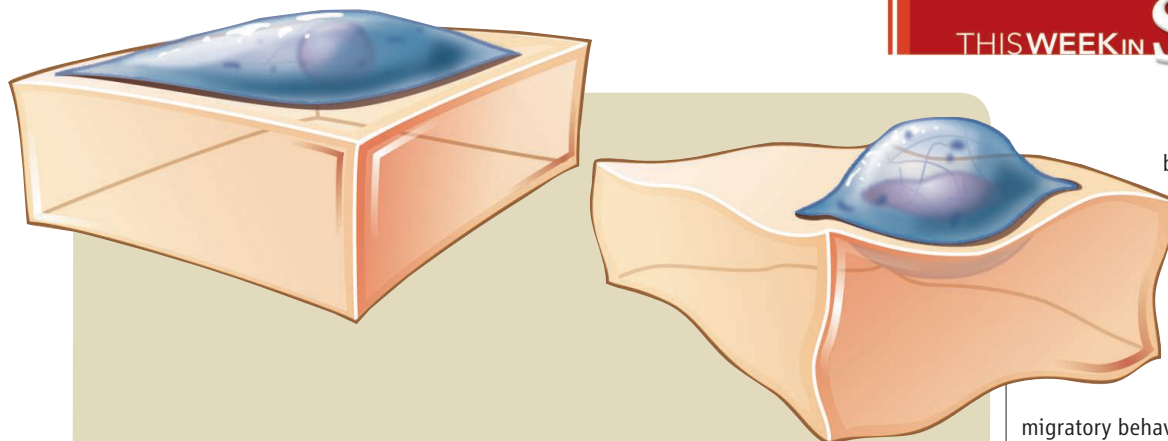
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birds within a group of migrating cranes significantly decreased the deviations the flock took from a straight line migration path. The lack of evidence for a genetic component indicates that social learning dominates any innate capacity in developing migratory behavior.

Lamins and Tissue Stiffness

Microenvironment can influence cell fate and behavior; for example, extracellular matrix (ECM) stiffness increases cell proliferation, and ECM rigidity induces disorders in tissue morphogenesis by increasing cell tension. **Swift et al.** (p. 975; see the Perspective by **Bainier and Weaver**) used proteomics to identify molecules that are mechanical sensors for tissue elasticity in the nucleus and discovered that expression of lamin-A levels apparently functions as a “mechanostat.”

Strategies for Metal-Organic Frameworks

Metal-organic frameworks are porous materials that can exhibit very high surface areas that have potential for applications such as gas storage and separation, as well as catalysis. **Furukawa et al.** (p. 974) review the structures devised so far and discuss the design strategies that allow families of materials to be synthesized and modified with similar framework topology but vary in pore size and type of functional groups present on the linkers.

A Uranian Trojan

Bodies that share their orbit with that of a planet and that trail or lead the planet by 60° are called Trojans. Based on data from the Canada-France-Hawaii Telescope, **Alexandersen et al.** (p. 994) have found an object shadowing Uranus that is predicted to remain a Trojan for at least 700,000 years and to stay in co-orbital motion for around one million years before escaping.

Depression and the Habenula

The lateral habenula (LHb) appears to have a role in depression. However, the underlying mechanisms are poorly understood, and by using multiple rodent models of depression, **Li et al.** (p. 1016) identified a signaling pathway and associated neuronal adaptations in which the enzyme β CaMKII was selectively up-regulated in

the LHb. Manipulations that enhanced β CaMKII levels increased depression-related phenotypes, and RNA interference of β CaMKIIb blunted depression. Enhanced β CaMKII levels in the habenula promoted excitatory synaptic transmission on these neurons and increased action potential firing mediated by an up-regulation of a specific subtype of glutamate receptors.

Ice Lubricant

The Greenland and Antarctic ice sheets both possess hydrological systems that allow water accumulating from the melting of surface ice to be transported to the base of the ice sheet. If that water, when it reaches the ice-bedrock interface, is distributed over large areas, it will lubricate rapid ice sheet flow toward the sea. **Bamber et al.** (p. 997) report the existence of a large, 750-km-long subglacial canyon in northern Greenland, which may act as a channel for the transport of basal meltwater to the margin of the ice sheet and thus influence overall ice sheet dynamics.

Follow the Leader

How birds migrate between wintering and breeding grounds, often over thousands of kilometers through difficult conditions, remains mysterious. The recovery of North American Whooping Cranes by release of captive-reared birds trained to migrate by following aircraft provided an opportunity for **Mueller et al.** (p. 999; see the cover) to analyze 8 years of data for individual birds. The presence of older

Egg WAVE1

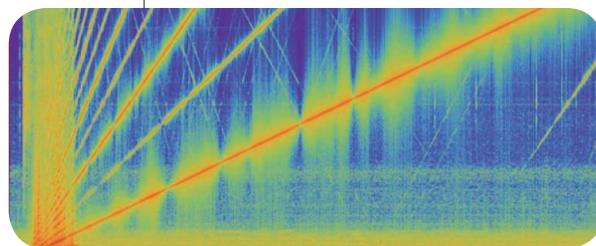
Eggs not only activate sperm nuclei for normal development but also reprogram transplanted somatic nuclei. In addition to its well-established cytoplasmic role in actin organization, **Miyamoto et al.** (p. 1002) discovered that the Wiskott-Aldrich syndrome protein family member 1 in oocytes cooperates with transcriptional machineries in the nucleus to activate previously silenced genes.

Cilium Conundrum

The cilium has emerged as the antenna of eukaryotic cells, having numerous functions in sensory reception and developmental signaling. Several disorders, such as polycystic kidney disease, are the result of compromised cilia structure. **Bhogaraju et al.** (p. 1009) elucidate how the intraflagellar transport machinery recognizes tubulin, a ciliary cargo that is integral to cilium maintenance and formation and is constantly turned over at the cilium tip.

Hydrogel Stretch

A range of stretchable, conductive materials can be made either by making an electrical conduc-

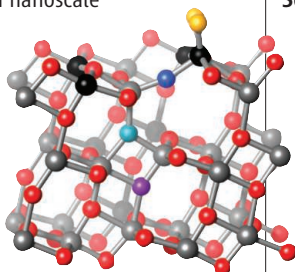


tor more stretchable or by adding an electrical conductor to a stretchable material. **Keplinger et al.** (p. 984; see the Perspective by **Rogers**) have added to the possibilities of an alternative stretchable ionic conductor based on a hydrogel material used to make deformable devices that are fully transparent to light over the visible spectrum and that can withstand high voltages and high frequencies.

Additional summaries

Oxide Chemistry Below the Surface

Although metal oxides, such as titanium dioxide (TiO_2), are used for catalytic oxidation reactions and photocatalysis, the O_2 does not react directly with substrates. Vacancies in the surface region of the TiO_2 rutile phase can transfer a negative charge to adsorbed O_2 to create more reactive species. By contrast, in anatase—the phase associated with nanoscale TiO_2 particles—subsurface vacancies form. **Setvin et al.** (p. 988) used a scanning tunneling microscopy tip to pull these vacancies to the surface in a niobium-doped anatase crystal and followed the transformation of adsorbed O_2^- into a peroxo species and a bridging O_2 dimer.



Burden of Poverty

Lacking money or time can lead one to make poorer decisions, possibly because poverty imposes a cognitive load that saps attention and reduces effort. **Mani et al.** (p. 976; see the Perspective by **Vohs**) gathered evidence from shoppers in a New Jersey mall and from farmers in Tamil Nadu, India. They found that considering a projected financial decision, such as how to pay for a car repair, affects people's performance on unrelated spatial and reasoning tasks. Lower-income individuals performed poorly if the repairs were expensive but did fine if the cost was low, whereas higher-income individuals performed well in both conditions,

as if the projected financial burden imposed no cognitive pressure. Similarly, the sugarcane farmers from Tamil Nadu performed these tasks better after harvest than before.

The Galaxy Center in X-rays

At the center of our Galaxy there is a black hole 4-million-fold more massive than the Sun.

Wang et al. (p. 981; see the Perspective by **Schnittman**) report x-ray data on the accretion flow around this supermassive black hole, revealing how it interacts with its surroundings. The data rule out the possibility that the quiescent (that is, flare-free) x-rays observed are produced by coronal emission from a population of stars at the center of the Galaxy and also rule out the possibility that there is a pure radiatively inefficient accretion flow with no outflows.

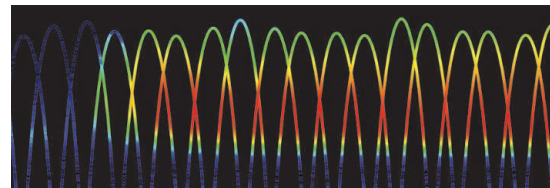
Pluripotency Control

The transcription factors Pou5f1/Oct4, Sox2, and Nanog play central roles in pluripotency control in mammalian embryonic stem (ES) cells. The evolution of the pluripotency regulatory network and its roles during early development of non-mammalian vertebrates is unknown.

Leichenring et al. (p. 1005, published online 15 August) show that in zebrafish embryos, Pou5f1 controls priming and transcriptional activation of the first zygotically expressed genes. This mechanism for transition from the transcriptionally silent cleavage stage to the transcriptionally active blastula stage may have evolved to control the prolonged cell pluripotency state in mammalian early development and ES cells, establishing a link between zygotic gene activation and pluripotency control.

Local Acceleration

How the electrons trapped in Earth-encircling Van Allen radiation belts get accelerated has



been debated since their discovery in 1958. **Reeves et al.** (p. 991, published online 25 July) used data from the Van Allen Radiation Belt Storm Probes, launched by NASA on 30 August 2012, to discover that radiation belt electrons are accelerated locally by wave-particle interactions, rather than by radial transport from regions of weaker to stronger magnetic fields.

Moving Bricks with MraY

Peptidoglycan, the building brick of bacterial cell walls, is synthesized in the cytoplasm and must be transported across the cell membrane. To achieve this, it is attached to a carrier lipid by the integral membrane protein MraY. MraY is targeted by natural antibacterials and is a promising antibiotic target. **Chung et al.** (p. 1012) report the crystal structure of MraY at 3.3 Å resolution. The structure, together with mutational mapping, outlines the location of the active site and provides interesting hints for how the enzyme binds the substrate and catalyzes attachment to the carrier lipid.



Marcia McNutt is Editor-in-Chief of *Science*.

Accelerating Ocean Exploration

LAST MONTH, A DISTINGUISHED GROUP OF OCEAN RESEARCHERS AND EXPLORERS CONVENED IN Long Beach, California, at the Aquarium of the Pacific to assess progress and future prospects in ocean exploration. Thirteen years ago, U.S. President Clinton challenged a similar group to provide a blueprint for ocean exploration and discovery. Since then, the fundamental rationale has not changed: to collect high-quality data on the physics, chemistry, biology, and geology of the oceans that can be used to answer known questions as well as those we do not yet know enough to pose, to develop new instruments and systems to explore the ocean in new dimensions, and to engage a new generation of youth in science and technology. Recently, however, exploration has taken on a more urgent imperative: to record the substantial changes occurring in largely undocumented regions of the ocean. With half of the ocean more than 10 kilometers from the nearest depth sounding, ecosystem function in the deep sea still a mystery, and no first-order baseline for many globally important ocean processes, the current pace of exploration is woefully inadequate to address this daunting task, especially as the planet responds to changes in climate. To meet this challenge, future ocean exploration must depart dramatically from the classical ship-based expeditions of the past devoted to mapping and sampling.

As a first step, future exploration should make better use of autonomous platforms that are equipped with a broader array of in situ sensors, for lower-cost data gathering. Fortunately, new, more nimble, and easily deployed platforms are available, ranging from \$200 kits for build-your-own remotely operated vehicles to long-range autonomous underwater vehicles (AUVs), solar-powered autonomous platforms, autonomous boats, AUVs that operate cooperatively in swarming behavior through the use of artificial intelligence, and gliders that can cross entire oceans. New in situ chemical and biological sensors allow the probing of ocean processes in real time in ways not possible if samples are processed later in laboratories.

Exploration also would greatly benefit from improvements in telepresence. For expeditions that require ships (very distant from shore and requiring the return of complex samples), experts on shore can now "join" through satellite links, enlarging the pool of talent available to comment on the importance of discoveries as they happen and to participate in real-time decisions that affect expedition planning. This type of communication can enrich the critical human interactions that guide the discovery process on such expeditions.

Words such as "crowd sourcing," "crowd funded," and "citizen scientist" are nowhere to be found in the President's Ocean Exploration Panel report of 2000, but at the Long Beach meeting, intense excitement revolved around growing public engagement in many aspects of ocean exploration through mechanisms that did not exist 13 years ago. However, there is not yet a body of experience on how to take advantage of this new paradigm on the scale of a problem as large as ocean exploration. For example, what tasks are most suitable for citizen scientists, and how can they be trained efficiently? Can the quality control of their work be automated? Can crowd-sourced tasks be scheduled to avoid duplication and gaps?

Should any region of the ocean receive priority? Although the southern oceans are still largely unexplored, and coral reef hot spots for biodiversity are gravely imperiled by ocean warming and acidification, there was much support by Long Beach participants for prioritizing the Arctic, a region likely to experience some of the most extreme climate change impacts. An ice-free ocean could affect weather patterns, sea conditions, and ecosystem dynamics and invite increases in shipping, tourism, energy extraction, and mining. Good decisions by Arctic nations on Arctic stewardship, emergency preparedness, economic development, and climate change adaptation will need to be informed by good science. Exploration of this frontier needs to happen now to provide a useful informational baseline for future decisions.

— Marcia McNutt

10.1126/science.1244099





ECOLOGY

About FACE

Globally, forests and their soils account for about twice as much carbon as is present in the atmosphere, and hence the response of forests to increasing atmospheric CO₂ is expected to be an important component of the global carbon cycle. However, compared to what is known from studies of small trees and herbaceous vegetation, in which elevated CO₂ has been shown to have a fertilizing effect on growth, there has been relatively little experimental research on mature forest stands. Using a 45-m canopy crane in a 100-year-old mixed deciduous forest in Switzerland, Bader *et al.* exposed tree crowns to 550 ppm CO₂ over 8 years and compared the ecosystem's response to that of control trees not exposed to elevated CO₂. Key plant traits, including litter production, radial stem growth, leaf biomass, and foliar nitrogen, were unaffected. However, there were several indicators of altered below-ground processes, including enhanced nitrification and nitrate leaching and reduced water uptake by roots. Hence, it appears that—at least for the trees in this mature forest stand—elevated CO₂ does not translate into increased carbon sequestration by the vegetation and that the consequences are more likely to manifest themselves in alterations in soil processes and nutrient cycling. — AMS

J. Ecol. **101**, 10.1111/1365-2745.12149 (2013).

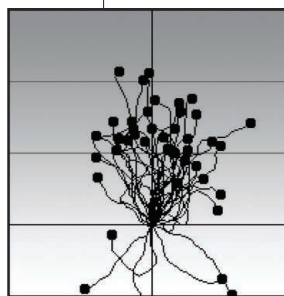
BIOMEDICINE

Model Therapies

If cellular signaling pathways were discrete and linear, controlling signals gone awry—like those from growth-promoting receptor tyrosine kinases often linked to cancer—would be straightforward. But these pathways form entangled and dynamic networks, and inhibiting signal transmission at one node, although successful in the short term, is often thwarted by regulatory mechanisms that keep cells healthy by rendering them robust to perturbations. Two groups have used a combination of mathematical modeling and experiments to identify strategies that may more effectively fight excess signaling by the ErbB family of receptors, which is associated with breast cancer. Kirouac *et al.* used their

model to search for combinations of two or three inhibitors that would overcome adaptive feedback and validated these effects in cell and animal models of cancer. Meyer *et al.* used a model, data from public databases, and their own experiments to identify a second receptor, AXL, which allowed cancer cells to resist the effects of ErbB receptor inhibitors. In this scenario, ligand-independent activating interactions between receptors of the ErbB family and AXL appeared to be crucial, suggesting that reducing receptor number or activity is more likely to be effective than treatments that target ligand-induced activation of the receptors. — LBR

Sci. Signal. **6**, ra68; ra66 (2013).



MOLECULAR BIOLOGY

Noncoding mRNAs

Chromatin insulators are protein complexes that act to insulate portions of the genome from the powerful and potentially disruptive effects of transcriptional enhancers, which can activate the expression of genes across vast stretches of DNA. This barrier function of insulators allows them to demarcate transcriptional domains in chromatin. The *Drosophila* gypsy insulator complex consists of three core proteins: suppressor of Hairy wing [Su(Hw)], modifier of mdg4 [Mod(mdg4)], and centrosomal protein 190 (CP190). RNA has also been implicated in gypsy insulator function, and Matzat *et al.* find that nine messenger RNAs (mRNAs) are specifically associated with the gypsy complex. Curiously, two of these interacting mRNAs code for the Su(Hw) and CP190 proteins themselves, a phenomenon not seen with other chromatin-associated proteins and their mRNAs. The expression of untranslatable versions of the Su(Hw) and CP190 mRNAs in larvae, which were already expressing wild-type Su(Hw) and CP190 mRNAs, resulted in the formation of supernumerary insulator bodies in the nucleus. They also increased the gypsy insulator enhancer-blocking activity in two marker genes that affect wing notching and eye color in adult flies. The insulator-targeted mRNAs may bind to the gypsy core complex through adaptor proteins. — GR

EMBO Rep. **14**, 10.1038/embor.2013.118 (2013).

CELL BIOLOGY

Neutrophil Knowhow

Chemotaxis involves the movement of cells along an attractant gradient and is important, for example, in the migration of neutrophils toward sites of infection. Chemotaxis requires both increased motility and sustained directionality. The molecular mechanisms involved in

directionality control are largely unknown. Working with mouse neutrophils, Kamakura *et al.* found that the directional movement of neutrophils was regulated via a heterotrimeric G protein signaling pathway. Chemoattractant binding to G protein-coupled receptors induced accumulation of the GDP-bound Gα_i subunit at the front of migrating neutrophils, where it recruited the conserved cell polarity protein mInsc. Neutrophils

lacking mInsc, although motile, were unable to stay the course and failed to stabilize pseudopods appropriately at the leading edge. — SMH

Dev. Cell **26**, 292 (2013).

CLIMATE SCIENCE

Which Emissions to Reduce?

A fierce debate is being waged about the relative merits of controlling the anthropogenic emissions of climate-affecting pollutants with long



atmospheric lifetimes, such as carbon dioxide, and those with shorter atmospheric lifetimes, such as methane or black carbon. Both classes of compounds have substantial radiative impacts, and arguments about which is more important to reduce have been made for each. By using a sophisticated global model, Smith and Mizrahi examined the potential climate effects of a strategy to mitigate the short-lived climate-forcing agents methane and black carbon and concluded that the likely benefits of such an approach are much more modest than had been suggested recently. Even assuming the maximally feasible reductions in emissions over the next decades, the rise in global average surface air temperature by the year 2050 would be only about one-half of the previous best estimate. Thus, a policy of focusing on reducing emissions of short-lived climate forcers, attractive to many because of its rapid effect and possibly easier and less costly implementation, may not be the silver bullet some have hoped it might be. — HJS

Proc. Natl. Acad. Sci. U.S.A. **110**, 10.1073/pnas.1308470110 (2013).

CHEMISTRY

Safer Cyanation

Cyanide (typically in an alkali salt) is such a well-known poison that many people may not realize how central it is to the production of pharmaceuticals and fine chemicals more generally.

Covalently bound to carbon, the CN group no longer manifests the same sort of toxic properties, but getting it there—particularly onto aromatic rings—can be a fraught process. This is not only on account of its danger in bulk quantities; another problem is its tendency to bind tightly to metal catalysts and deactivate them before the reaction is complete. Senecal *et al.* present a practical and highly versatile catalytic method for cyanation of aryl chloride and bromides. They combine a previously investigated, nontoxic cyanide source (potassium ferrocyanide) with a particular palladium precursor and phosphine ligand combination that manifests high activity and broad functional group tolerance in a dioxane/water mixture with acetate base. The reactions, which generally proceed within 1 hour at 100°C with <1% catalyst loading, show compatibility with OH and NH groups, esters, aldehydes (at lower temperature to suppress competing benzoin condensation), and sulfur and nitrogen heterocyclic frameworks. — JSY

Angew. Chem. Int. Ed. **52**, 10.1002/anie.201304188 (2013).

MATERIALS SCIENCE

Insensitive Superconductors

Superconductors are materials that perfectly conduct electricity below a transition temperature T_c , which generally depends on the density of the current carriers. The bilayer of the insulating cuprate compound La_2CuO_4 and the metal $\text{La}_{1.65}\text{Sr}_{0.45}\text{CuO}_4$ is an unusual system, where superconductivity has been shown to occur in a single copper-oxide plane. Wu *et al.* studied the dependence of T_c on carrier density in a system in which the Sr content of the doped layer $\text{La}_2\text{-xSr}_x\text{CuO}_4$, labeled x, was varied in tiny steps. The films were grown by molecular beam epitaxy with a small doping gradient, so that a single film, after lithographic patterning, yielded a combinatorial library of chemical compositions; this technique reduced sample-to-sample variations associated with experimental conditions. The authors measured more than 800 different compositions, covering the range of x from 0.15 to 0.47, and found that T_c remained virtually unchanged; in contrast, the Hall resistance, which reflects carrier density, varied by almost an order of magnitude over the same range. This unusual insensitivity of the transition temperature to carrier density presents a challenge to the theories of superconductivity in interfacial systems. — JS

Nat. Mater. **10**.1038/nmat3719 (2013).

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Right site? A Japanese panel suggests locating the ILC in a tunnel in the Kitakami Mountains.

announced on 23 August—if the proposed \$10 billion facility ever gets built. The ILC would be the follow-on to Europe's Large Hadron Collider.

Physicists in North America, Europe, and Japan are collaborating on a design, and Japan had proposed two possible sites: on the southern island of Kyushu; and in the northern Tohoku region, where engineers propose boring a 31-kilometer-long tunnel through the Kitakami Mountains. The Tohoku site is preferable because of its geology and infrastructure, the panel said. Backers also hope that the spot might make the ILC eligible for recovery funding from the 2011 tsunami, because most of the tunnel would be in Iwate Prefecture, which was battered by the disaster. But politicians will have to make that decision, warns Satoru Yamashita, a University of Tokyo physicist who chairs Japan's ILC Strategy Council. And Japan isn't likely to start the project, he adds, without international partners. <http://scim.ag/ILCsite>

FINDINGS

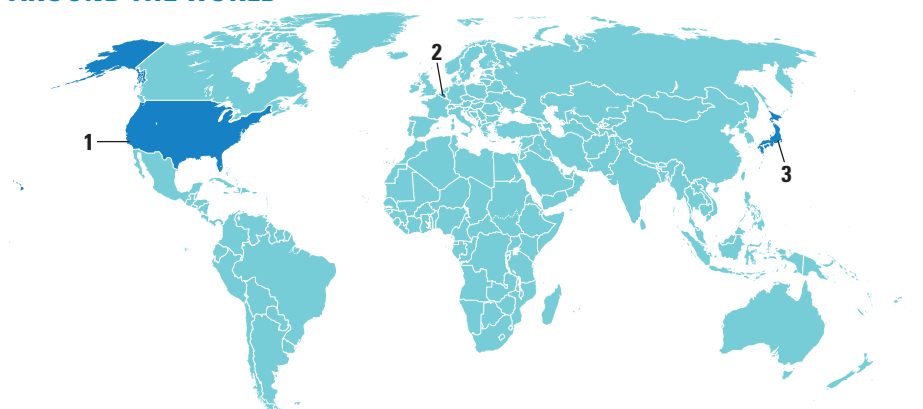
Learning Language in the Womb

As a fetus grows inside a mother's belly, it can hear sounds from the outside world—and can retain memories of them after birth, according to a study online this week in the *Proceedings of the National Academy of Sciences*.



The sound-processing parts of a fetus's brain become active in the last trimester of pregnancy, and sound carries fairly well through the mother's abdomen, says cognitive neuroscientist Eino Partanen of the University of Helsinki. He and his team outfitted babies with EEG sensors to look for neural traces of memories from the womb; hearing a repeated sound forms a memory, he says, which speeds up recognition of

AROUND THE WORLD



Los Angeles, California **1**

Genomics XPRIZE Cancelled

The XPRIZE Foundation has scrapped its \$10 million genomics challenge, set for next month, after attracting only two competitors to the sequencing contest.

First announced 7 years ago, the Archon Genomics XPRIZE was revamped in 2011 to offer \$10 million to the first team to sequence the entire genomes of 100 centenarians with high accuracy within 30 days—and for \$1000 or less apiece. But only two of the eight contenders in the



Off target. The XPRIZE Foundation called off its \$10 million genomics contest, launched in 2006.

original contest registered by the 31 May deadline—the company Ion Torrent, and George Church's lab at Harvard University.

On 22 August, XPRIZE CEO Peter Diamandis announced that the foundation was calling off the contest because it “was not incentivizing the technological changes” laid out by the XPRIZE board and the genomics prize's chair, Craig Venter. Companies are already sequencing human genomes in a few days for \$5000 and “are moving quickly towards the goals we set for the prize,” Diamandis wrote.

Church, who says he's spent “embar-

assing” amounts of money to prepare, calls the decision “a totally avoidable slap at innovators.” <http://scim.ag/Xprizegen>

Brussels **2**

Pesticidemakers Challenge E.U. Ban in Court

Two agrochemical companies have taken legal action against an E.U.-wide ban on three widely used neonicotinoid pesticides. In April, the European Commission introduced a 2-year moratorium on the compounds—clothianidin, imidacloprid, and thiamethoxam—following reports by the European Food Safety Authority that the substances pose an “acute risk” to honey bees essential to farming and natural ecosystems.

But this week, Bayer CropScience and Syngenta Crop Protection, two firms that manufacture and sell the pesticides in Europe, announced that they have brought two separate legal cases before the Court of Justice of the European Union earlier this month. “In suspending the product, [the European Commission] breached EU pesticide legislation and incorrectly applied the precautionary principle,” Syngenta's Chief Operating Officer John Atkin said in a statement.

It may take the court years to reach a decision. The legal cases have no suspensory effect, so the restrictions on the three chemicals will apply as planned from 1 December in all 28 E.U. member states.

Tohoku, Japan **3**

Panel Picks Possible Collider Site

A site near the northern end of Japan's main island is the best spot for the International Linear Collider (ILC), a Japanese panel

Random Sample

The Incredible Shrinking Springtail

Many organisms, from fish to earthworms, grow throughout their lives. But, when it gets hot, the already tiny springtail (*Folsomia candida*) dwindles in size. Graduate students Alexandre Peluffo and François Mallard from Ecole Normale Supérieure in Paris looked into the shrinking ability of the 2-millimeter-long, six-legged creature after noticing the relatively small average size of a group of springtails raised at high temperatures.



Shrunk. When the heat is on, the springtail gets smaller.

The researchers raised eight populations of springtails at 16°C then put half of the adults into chambers warmed to 26°C. Each time the animals molted over the 70-day test period, the researchers measured them. Different lines responded differently, indicating that shrinking was genetically controlled: None grew bigger, a few stayed the same size, and many got smaller by as much as 30%, the team reported last week at the annual XIV Congress of the European Society for Evolutionary Biology in Lisbon.

So what's the advantage of shrinking? "When temperature is high, metabolic rates increase and energy requirements increase," says Glauco Machado, an evolutionary biologist at the University of São Paulo in Brazil. "Small individuals survive better under these conditions." Because springtails live about 6 months, long enough to experience consistently warm or cool temperatures depending on the seasons, computer simulations showed that it was advantageous for them to shrink to match the temperature.

sounds and can be detected as a pattern of brain waves—even in a sleeping baby.

The team gave expectant women a recording to play several times a week during their last few months of pregnancy, which included a made-up word, "tatata." By the time the babies were born, they had heard the made-up word, on average, more than 25,000 times. When they were tested after birth, these infants' brains recognized the word, while infants in a control group did not. <http://scim.ag/wombwords>

Slower Warming Tied To Pacific Cooling

Although greenhouse gas concentrations continue to rise, over the last 15 years there has been a puzzling "hiatus" in the rise of global mean temperatures. Some scientists have suggested that this hiatus may be due to increasing amounts of aerosols, or to the solar activity minimum in 2009. But a new study in *Nature* has identified a different cause: variability in tropi-

THEY SAID IT

"One thing that I certainly read from viable sources is that a lot of the research that's being done—when you put your application in to get a grant, if you don't submit to the, you know, orthodoxy of climate change by the radical environmentalists, you're not going to get a grant."

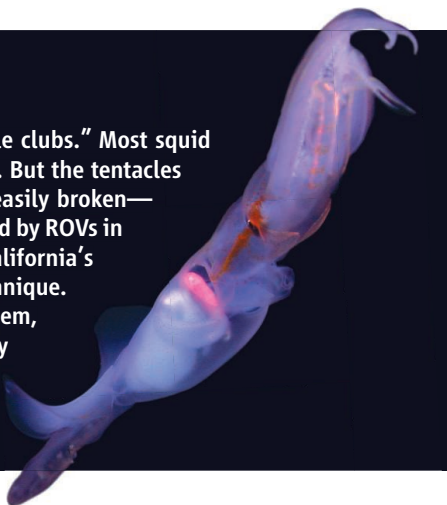
—Representative Mike Coffman (R-CO), responding to a League of Conservation Voters ad that portrays him as an "ostrich" on climate change issues.

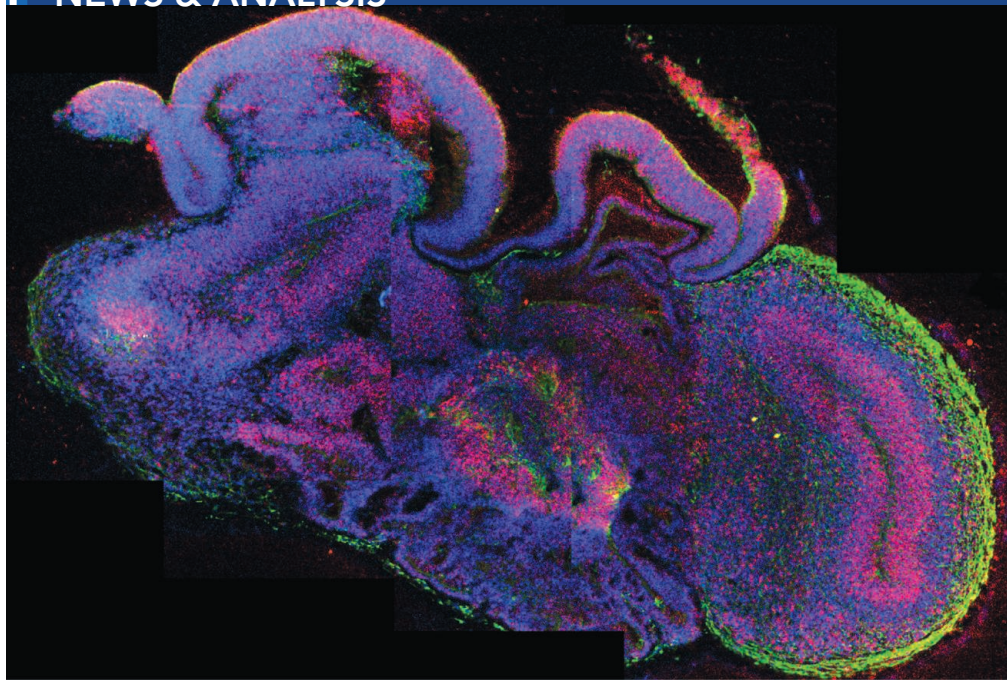
cal Pacific sea-surface temperatures.

Climatologist Yu Kosaka of the Scripps Institution of Oceanography in San Diego, California, and colleagues examined the effect of periodic cooling in the eastern equatorial Pacific Ocean using several different model experiments and compared these with the observed records. In one model, they incorporated only the increased greenhouse gas concentrations, aerosol concentrations, and solar cycle changes. In the second, they fixed these at 1990 levels, but included changes in sea-surface temperatures in the equatorial Pacific. In the third model, they incorporated both. That model, they found, best reproduced the observed records. Together, Kosaka says, the models suggest that a cooling trend in this patch of ocean from 2002 to 2012 was responsible for the mysterious hiatus. <http://scim.ag/warmpause>

Reeling Them In

Among a squid's most defining characteristics is its long pair of tentacles, or "tentacle clubs." Most squid extend and retract these tentacles—armed with suckers or hooks—to snatch their prey. But the tentacles of the deep-sea squid *Grimalditeuthis bonplandi* are unique: thin and unarmed, and easily broken—prompting scientists to wonder just how the animal captures its food. Now, video captured by ROVs in the Atlantic and North Pacific oceans, deployed by scientists led by Hendrik Hoving at California's Monterey Bay Aquarium Research Institute, reveal *G. bonplandi*'s unusual tentacle technique. Rather than rapidly extending and retracting its fragile tentacle stalks, it undulates them, which may serve to stimulate bioluminescence, produce a wake, or create low-frequency vibrations—any of which may attract small marine organisms and lure them to their fate, the team reports this week in the *Proceedings of the Royal Society B*.





Inside view. A cross section of a cerebral organoid shows neural stem cells (red) and neurons (green).

NEURODEVELOPMENT

Lab Dishes Up Mini-Brains

No bigger than apple seeds, the cell clusters are simply referred to as “cerebral organoids.” But that careful language in a paper in this week’s issue of *Nature* belies the excitement of many neuroscientists at what it reports: the growth from human embryonic stem cells of semiorganized knots of neural tissue that contain the rudiments of key parts of the human brain, including the hippocampus and prefrontal cortex.

“I find it amazing,” says Wieland Huttner, a neuroscientist at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, who was not involved in the study. “It’s not real brain—that’s clear. But I’m positively surprised that so many features are reproduced.” Developmental geneticist Madeline Lancaster and her colleagues, who grew the organoids in a Vienna laboratory, have already shown that they can use the organoids to probe how normal human brain development goes awry in a genetic brain disorder.

Left to their own devices in a lab dish, embryonic stem (ES) cells will differentiate into a menagerie of tissues: beating heart cells, neurons, even hair and teeth. The trick for scientists has been to harness that potential, coaxing the cells to grow into the kinds of tissues they want to study or use for a therapy.

Developmental biologists know that neural tissue is a sort of default fate for differentiating embryonic cells, and researchers

have been able to grow a variety of specific neural cell types from ES cells. But the level of cellular organization seen in this latest brain-in-a-dish paper is a significant step forward, says Magdalena Götz, who studies neurodevelopment at the Ludwig Maximilian University of Munich in Germany.

Lancaster’s work took place in the lab of Jürgen Knoblich, a developmental geneticist at the Institute of Molecular Biotechnology of the Austrian Academy of Science in Vienna. It exploits what Knoblich calls the “absolutely enormous self-organizing capacity of developing human cells. If you just leave them alone and provide a medium that is supportive enough, they do things on their own.” It also builds on work by Yoshiki Sasai of the RIKEN Center for Developmental Biology in Kobe, Japan. In 2008, he and his colleagues reported that mouse and human ES cells in culture could spontaneously form cell layers that resemble the cortical layers in the brain.

Lancaster, a postdoctoral researcher in Knoblich’s lab, was trying to culture early neural tissue to better understand how and when developing brain cells switch from proliferation—making more of themselves—to differentiation—making more mature cell types, which don’t continue to divide. Lancaster started with techniques developed by Sasai and others that shepherd dividing stem cells toward a neural fate, but she was

also intrigued, she says, by the “mini-guts” that another research team had grown in droplets of Matrigel, a gelatinous protein mixture that can help cells grow in three dimensions. So she embedded clusters of stem cell–derived neural cells in a droplet of the material (see diagram).

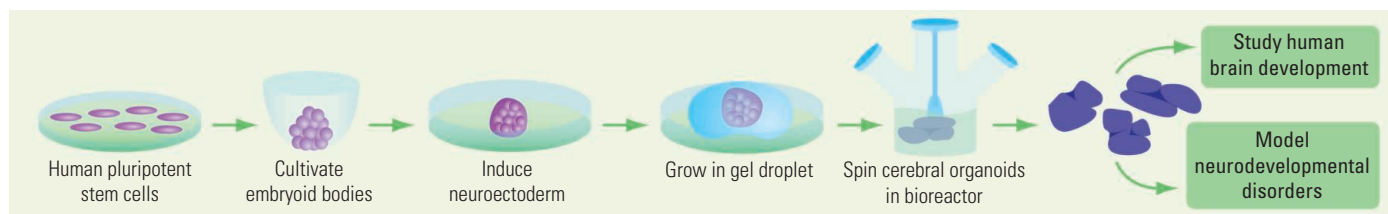
The Matrigel droplets freed the cell clusters to grow larger and develop more complex structures, without any further coaxing. To increase the availability of oxygen and other nutrients to the inner layers of the structures, Lancaster put the Matrigel droplets into a slowly rotating bioreactor, which gently shakes them.

Within a few weeks, Lancaster says, she noticed darker pigmented patches on some of the cell clusters. On closer inspection, she recognized the rudiments of eye tissue, a sign that more complex structures might be forming. When she described the data at a lab meeting, Knoblich says, “I was completely blown away. I couldn’t sleep that night.”

When lab members looked inside some of the cerebral organoids, they found structures that resemble the choroid plexus (the cavity in the brain that produces cerebrospinal fluid), the cerebral cortex (the brain’s outermost layer), and retinal tissue. More detailed staining showed evidence that after 16 days of development, the organoids had what resembles forebrain, midbrain, and hindbrain regions. The team also found molecular markers for a variety of more specialized regions—including the outer subventricular zone (OSVZ), a feature of human, but not mouse, brains. Organoids grown from mouse ES cells did not develop an OSVZ region.

The resemblance to a real brain only goes so far. The organoids do not have any blood vessels, so cells at their core die. They reach their maximum size—about 3 millimeters in diameter—after 2 to 3 months, Lancaster says, and after 4 months they don’t develop any new cell types. However, the cell clusters can apparently survive indefinitely in the bioreactor; the oldest ones have been in culture for nearly a year, the researchers report.

The cerebral organoids may shed light on human brain diseases that are difficult to study in mice or other animals. For



example, the scientists used the structures to study microcephaly, a neurodevelopmental disorder in which the head and brain end up much smaller than normal. Rather than starting with ES cells, they took cells from a person with a particular form of the disorder and “reprogrammed” them into so-called induced pluripotent stem (iPS) cells. Mice are a poor model for that form of microcephaly; the specific genetic mutation responsible results in mice with brains that are only slightly smaller than normal. In contrast, organoids derived from the patient’s iPS cells were shrunken, and Knoblich’s team

found a clue to why. Certain precursor cells were maturing earlier than normal, bringing tissue growth to a halt prematurely.

The organoids are probably not yet useful for studying more complex neurodevelopmental conditions such as autism or schizophrenia, because those conditions involve more mature cells and complex cell connections. Lancaster and Knoblich also note that each organoid develops distinctively, resulting in significant differences in composition and structure that make it hard to do controlled experiments.

The researchers are working on ways to

Brain gain. The differentiation process from pluripotent cells to organoid takes about 3 weeks.

grow more consistent organoids—and to incorporate some sort of vascular system so that the cell clusters can grow bigger and presumably develop further. They hope that additional teams will take up and improve the method. Götz and others says they plan to do just that. “For studying the cerebral cortex, this is the best model so far,” she says. “People will use it, and time will tell how useful it is.”

—GRETCHEN VOGEL

CELL BIOLOGY

NIH Effort Gambles on Mysterious Extracellular RNAs

The versatility of RNA is legendary. Inside the cell it transfers genetic information, acts as an enzyme, and regulates genes. In plants and nematodes, short RNA sequences also act like hormones, carrying messages between cells. Now, a \$17 million program of research grants, announced this month by the U.S. National Institutes of Health (NIH), aims to determine whether extracellular RNA (exRNA) has a similar communication role in people—and whether it can be harnessed for diagnosis and treatment of human diseases.

“This is an effort to get a new field going,” says Christopher Austin, director of the National Center for Advancing Translational Sciences (NCATS), one of the five NIH centers and institutes participating in the 5-year exRNA initiative.

Conventional wisdom long held that the different varieties of RNA molecules toil mainly within the cells that make them. For more than a decade, however, researchers have seen signs that cells emit RNA molecules that travel throughout an organism and alter the activity of target cells. So far, says molecular biologist John Mattick of the Garvan Institute of Medical Research in Sydney, Australia, scientists have accrued

“unassailable” evidence that plant and nematode cells communicate through exRNA.

In humans and other mammals, exRNAs abound in blood, tears, saliva, and every other body fluid. Among the molecules wending through our bodies are messenger RNAs that carry the instructions for making proteins and microRNAs that fine-tune gene activity. The extruded RNA is typically enclosed in membrane-bound capsules such as exosomes (*Science*, 24 June 2005, p. 1862) or accompanied by protein or lipid bodyguards, which protect it from RNA-destroying enzymes.

But definitive data that such exRNAs influence recipient cells have been missing for mammals. “It hasn’t been convincingly shown that a small RNA can be active when it enters a different cell,” says molecular biologist Michael McManus of the University of California, San Francisco.

Many of the 24 newly funded projects are probing practical uses for exRNA. Some researchers will assess whether exRNAs can serve as biomarkers—molecular indicators that help doctors diagnose illnesses or identify which people are susceptible. A group led by cardiologist Jane Freedman of the University of Massachusetts Medical School in

Worcester, for instance, will sift blood samples from nearly 3000 participants in the famous Framingham Heart Study in hopes of learning whether certain exRNAs foretell cardiovascular disease. Even if they are just junk spit out by cells, the molecules may have some diagnostic value.

Other projects will investigate whether exRNA can be harnessed to fight diseases such as cancer, Huntington’s disease, and multiple sclerosis. For example, cancer biologists Thomas Schmittgen and Mitch Phelps, of Ohio State University, Columbus, are working to engineer cells to manufacture exosomes that home in on the liver and deliver a specific microRNA that stymies the growth of tumor cells.

McManus is teaming up with plant biologist Olivier Voinnet of the Swiss Federal Institute of Technology in Zurich and colleagues to try to settle the question of whether exRNAs have a biological role in mammals. Voinnet was one of the first researchers to uncover evidence for exRNA signaling in plants more than 15 years ago. No one has so far presented comparable data for humans, he says, but he and his colleagues are willing to be convinced that we use this RNA communication channel. “We are ‘positively skeptical’ about the whole issue,” Voinnet says.

—MITCH LESLIE

“This is an effort to get a new field going.”

—CHRISTOPHER AUSTIN, NCATS



The more the scarier. Like many bat species, Egyptian fruit bats form large groups, which may partly explain why they are a reservoir for Ebola and several other viruses.

and on to humans, killing more than 100 people. The most likely predecessor to SARS, which killed 775 people in 2002 and 2003, was identified in horseshoe bats in China. Investigations have also pointed to bats as the natural reservoir for the Ebola and Marburg viruses and for a whole list of more obscure agents, with names like Kasokero, Duvenhage, and Menangle.

The viruses come from many different families. SARS and MERS are coronaviruses; rabies, Duvenhage, and bat lyssavirus belong to the rhabdovirus family; whereas Nipah and Hendra are Paramyxoviruses. But they do have a few things in common: They are usually RNA viruses that replicate outside the cell's nucleus, and they seem to have high fatality rates in humans. The routes of infection vary. Bites can transmit rabies, but more often viruses spread when bat droppings infect an intermediate host that lives closer to humans.

The frequent spillovers could be largely a numbers game, Epstein says. With more than 1300 species—roughly one-fifth of all mammalian species—it's "not surprising" that bats cause a sizable share of the newly emerging diseases as well, he says.

New numbers suggest that statistics can't be the full explanation, however. Earlier this year, Angela Luis, a biologist at Colorado State University, Fort Collins, published an analysis comparing bats with another huge mammalian group, the rodents. Both harbor nasty viruses, are evolutionarily old, display a wide range of body sizes, and have some hibernating species. Sifting through the literature, Luis and her colleagues identified 68 viruses in rodents that can also cause disease in humans, versus only 61 in bats. But there are twice as many rodent species, so relatively speaking,

bats appear to be a more frequent source of emerging infections.

Even if bats unleash more zoonoses than other animals, that doesn't mean there is anything special about their physiology, Epstein says. Bats occur on every continent except Antarctica, and some species migrate over hundreds of miles; species also intermingle, for instance while roosting in caves. As a result, they may be exposed to more viruses than other mammals. Some species form

EMERGING INFECTIOUS DISEASES

Link to MERS Virus Underscores Bats' Puzzling Threat

Last week's announcement that a fatal new virus disease in the Middle East may have originated in bats has focused new attention on an enduring enigma: What is it about bats? A frightening litany of recently arrived human diseases—including Nipah virus, Hendra virus, severe acute respiratory syndrome (SARS), and now Middle East respiratory syndrome (MERS)—came from bats. Is there something special about these mammals that turns them into flying repositories of pathogens?

"That's the million dollar question," says Jonathan Epstein, a veterinary epidemiologist at the EcoHealth Alliance in New York City who collected samples from bats in Saudi Arabia for last week's paper in *Emerging Infectious Diseases*. Interest in the topic is huge, says veterinary pathologist Gudrun Wibbelt of the Leibniz Institute for Zoo and Wildlife Research in Berlin. "It's come to the point where you just have to add those three words 'Are bats special?' to a paper and it will get published," she jokes.

Some scientists do think bats are exceptional; they have invoked everything from hibernation and echolocation to peculiarities of bats' immune systems to explain why the Chiroptera, as their order is known, could pose a greater threat than others. But other researchers don't see the need for explana-

tions beyond bats' numbers and lifestyle.

The latest finding didn't come as a big surprise. Scientists already suspected that the MERS coronavirus came from bats, as they had found several closely related viruses in other bat species. In the new paper, a team led by Columbia University virologist Ian Lipkin reports finding an RNA fragment in the feces of an Egyptian tomb bat that matched the MERS virus exactly. They didn't find the virus itself, and the snippet was only 182 nucleotides long—but it made the species a prime suspect as the MERS reservoir (<http://scim.ag/MERSbats>).

It's only the latest example in a growing list. Bats have been known to carry the rabies virus for 60 years, but in the past 2 decades, many new diseases were traced back to them as well. Hendra virus, which first popped up in Australian horses and people who worked with them in 1994, was later found to originate in fruit bats; it has since caused numerous outbreaks and killed at least four humans. A surveillance program to track it led to the discovery of another deadly pathogen, the Australian bat lyssavirus.

In 1998, yet another newcomer, Nipah virus, jumped from bats to pigs in Malaysia,

"We think there is something very unusual going on in bats."

—LINFA WANG,
AUSTRALIAN ANIMAL
HEALTH LABORATORY

dense populations of thousands or millions of individuals—a mecca for viruses.

There are other factors: Bats are long-lived—some can live up to 40 years—which gives them a higher chance of transmitting a virus that they're persistently infected with. They likely first appeared about 50 million years ago, so viruses that evolved with them may be using very old, conserved receptors to gain access to cells, which might make the jump to other mammalian species easier. Another hypothesis holds that hibernation could make animals more susceptible to infections, but Luis found the opposite in her analysis: Hibernating species had slightly fewer viruses. Some scientists have even hypothesized that generating echolocation sounds—which come out through the mouth or the nose—creates fountains of droplets that help spread viruses.

Linfa Wang, who heads the “bat pack,” a research team at the Australian Animal Health Laboratory in Geelong, thinks that bat numbers and behavior can't explain it all. “We think there is something very unusual going on in bats,” he says. At conferences, proponents of these two viewpoints regularly clash, Wang says: “It's become a bit of a ritual.”

He thinks that the answer lies in bats' immune systems. Most viruses have been isolated from healthy bats, and researchers speculate that bats may have a remarkable capacity to play host to viruses for long periods of time while suffering few or no symptoms. Earlier this year, Wang and colleagues presented evidence for that idea in a paper describing the first two bat genomes—one from a small, insect-eating echolocating species, the other from a big, fruit-eating flying fox (*Science*, 25 January, p. 456). Strikingly, both species lacked a chunk of the genome containing genes involved in sensing microbial DNA and initiating an inflammation response. In addition, genes involved in repairing DNA damage appeared to evolve faster in bats than in other mammals.

Wang's hypothesis is that during flight, bats have to dramatically increase their heart rate and energy consumption. This releases metabolites that damage DNA, he argues, so evolution came up with special DNA repair mechanisms. But because the immune system uses some of the molecules involved in DNA repair, the result was a “robust but primitive system,” that left bats more vulnerable to infections but caused less of the damage typically created when a strong immune system fights a pathogen. Better DNA correction might also explain bats' longevity. “I think Linfa is on to something here,” Lipkin says.

Other scientists are unconvinced. Wibbelt, who has dissected hundreds of dead bats, says that many of them may well have died of viral infections. So little is known about bats' immune systems that much of the debate is pure speculation, she says. “We've learned a few new things about bat immunity, but at a frustratingly slow pace,” adds Andrew Dobson, an infectious diseases ecologist at Princeton University. One problem is that bats are not easy to study; many species are endangered and lab colonies are hard to set up.

Many ecologists think that the biggest factor in the viral spillover from bats is their proximity to people. The tomb bats linked to MERS, for instance, often live in old, abandoned buildings. People could become infected after kicking up some soil or dust contaminated with feces and inhaling it,

Epstein says. And as humans encroach on bat habitats, such interactions may become more common. “The principal ecological problem is that bat habitat is being destroyed and they are increasingly forced to roost in fruit and shade trees that are close to human dwellings,” Dobson says.

Many conservation biologists don't like to hear about new pathogens found in bats, because it could undermine efforts to preserve them, Epstein says: “They feel every discovery implicates bats in a bad way.” But if dwindling habitats are causing outbreaks, that's actually an argument to take conservation more seriously, he says. Bats may turn out to carry more, and more dangerous, viruses than other wildlife, Epstein says, “but how these viruses manage to spill over: That's on us.”

—KAI KUPFERSCHMIDT

PLANETARY SCIENCE

Taking the Life Out of Titan

Is Saturn's big, haze-shrouded moon Titan dead or alive? Many planetary geologists say alive. Radar images from the Cassini spacecraft show what look like volcanoes thought to spew icy “lavas” and pump methane into Titan's atmosphere. But other Cassini data have led a group of planetary physicists to a different view. They report this week that Titan appears to be encased

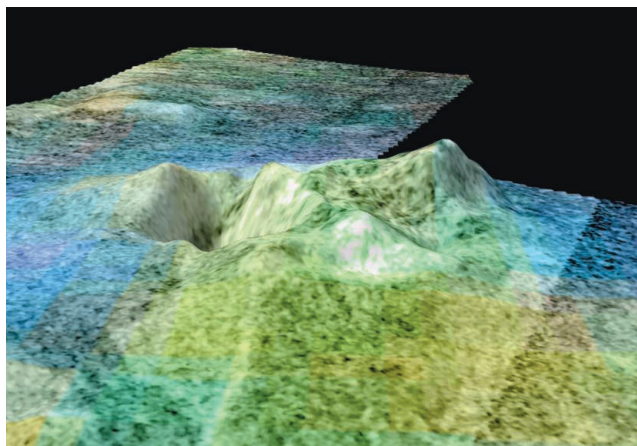
its canyons and fill its lakes—may be dwindling. Titan may be drying out.

In this week's issue of *Nature*, planetary physicist Douglas Hemingway of the University of California, Santa Cruz (UCSC), and his colleagues describe how they combined measurements of Titan's bumpy shape and its gravity to probe its icy interior. The Cassini spacecraft, which has been orbiting

Saturn since 2004, has made repeated close flybys of Titan. Many times, researchers monitored Cassini's radio signal frequency for Doppler shifts as Titan's lumpy gravity field accelerated the spacecraft off its expected track. To judge Titan's topography, they operated Cassini's radar as an altimeter and also derived elevations from overlapping radar images.

A funny thing turned up when the data were combined. “Where there is high topography, the gravity is lower than the surroundings,” says co-author Francis Nimmo of UCSC. And vice versa: Where topography is low, gravity is high. “That's very odd,” Nimmo notes. Ice piled high above an otherwise uniform interior should produce stronger gravity, not weaker.

The group concludes that beneath high



A singular volcano? Geologists agree that Titan's Sotra Facula (false color, exaggerated in height by 10 times) is a 1450-meter-high ice volcano, but planetary physicists doubt that many more will be found.

in a shell of rock-hard ice tens of kilometers thick—perhaps too rigid to allow ice magma to rise from the depths.

If they are right, the “volcanoes” are something else entirely, Titan is geologically dead, and the moon's store of atmospheric methane—the source of the rains that cut

spots, ice extends down into the water ocean thought to underlie Titan's outer shell of ice. Because ice is less dense than liquid water, the inferred deep ice keel reduces gravity despite the overlying high topography. But if that's the case, the group argues, the ice shell must be rigid enough and thick enough to keep the buoyant keel from floating the ice shell even higher, resulting in more surface relief than is observed. So the group concludes that Titan's ice shell is quite rigid from the surface down to at least 40 kilometers.

A thick, rigid ice shell "seems to be a robust result," Nimmo says. If so, he asks, how could icy magma from Titan's interior break through? "I'm a bit reluctant to rule out [volcanism] entirely, but it makes it substantially more difficult to argue it is happening."

Geologists, however, say their pictures don't lie. A feature in Cassini radar imaging

called Sotra Facula "is a cryovolcano," says planetary geologist and Cassini team member Ellen Stofan of Proxemy Research in Rector-town, Virginia. "It does everything a volcano is supposed to do." It rises to a peak, has a central depression resembling a volcanic caldera, and is surrounded by deposits that look as if they flowed down from the caldera.

Such methane-laden lavas, flowing from volcanoes all across Titan, could explain how the moon's atmosphere remains rich in methane even though solar ultraviolet continuously destroys it. Without volcanism, the methane may have been a one-time addition to the atmosphere in Titan's midlife that will eventually dwindle away.

Of course, the planetary physicists could be wrong. There are "fairly large uncertainties" with the gravity and topography data, points out planetary physicist David

Stevenson of the California Institute of Technology in Pasadena. "They've gone after the simplest explanation. It might be right, but I'm uneasy." Then again, Sotra Facula is the only cryovolcano about which the geologists are certain. "On all of Titan, we have this one feature we agree is a cryovolcano," Stofan says. "That's not a lot."

The Cassini mission is scheduled to run into 2017, but it may not settle the debate. There just aren't enough close flybys left to significantly improve the gravity mapping, Stevenson says. But geologists are just starting to take a close look at Titan's vast, smooth plains, which may be the frozen outpourings of eruptions whose lavas were too fluid to build a volcanic peak. If a careful look shows that the plains are volcanic, Titan might spring back to life.

—RICHARD A. KERR

ARCHAEOLOGY

European Hunter-Gatherers Dined on Domestic Pigs

About 8500 years ago, Near Eastern farmers began moving into Europe, a continent long peopled by hunter-gatherers. What went on between the two groups in their centuries of coexistence—and how did the natives react to this strange new way of life? Now, DNA from ancient pigs suggests that fishing peoples in Germany may have adopted at least one new custom: keeping domesticated pigs acquired from their neighbors.

If the claim holds up, it suggests that farming spread through locals adopting the technology, as well as farmers moving into new territory—processes that have often been seen as opposed to each other (*Science*, 27 April 2012, p. 400). The finds are a "dead giveaway for some interaction between farmers and late hunter-gatherers," says Wolfgang Haak, a paleobiologist at the University of Adelaide in Australia. Although others caution that alternative explanations are possible, Princeton University anthropologist Peter Bogucki thinks the study demonstrates "the porosity of the borderland between foragers and farmers."

In the paper, published online this week in *Nature Communications*, a team led by molecular biologist Almut Nebel of Christian-Albrechts University in Kiel, Germany, analyzed pig remains from sites of the Ertebolle people, semisedentary fishers and hunt-

ers who lived in Scandinavia and Germany between about 7500 and 6000 years ago. The team also studied pigs from sites further south in Germany that are associated with a farming culture, the Linienbandkeramik (LBK) people, who raised wheat, barley, and other crops; kept pigs, cattle, and sheep; and coexisted with the Ertebolle for at least 700 years. (Pigs were first domesticated at least

from ancient DNA in the bones, including the sequence of the melanocortin 1 receptor (MC1R), which helps determine coat color. Wild boars are usually gray, but the MC1R sequence in a few of the remains suggested that the pigs resembled modern spotted breeds. The team reached firm conclusions about 14 animals, finding that seven were domesticated. Four—including the oldest, dated to about 6600 years ago—came from two Ertebolle sites, and three came from two LBK sites.

Nebel and her colleagues suggest that the Ertebolle people either traded with farmers or captured escaped domestic pigs. LBK pottery found at Ertebolle sites suggests contact, so direct interaction "is a more likely scenario," she says. If so, these hunter-gatherers may have adopted farming behavior—such as keeping a few pigs in a pen.

Archaeologist Peter Rowley-Conwy of Durham University in the United Kingdom cautions that domesticated pigs may have escaped from farmers and bred with local wild boar, which were then

hunted by the Ertebolle people. Nebel's team concedes that the hunter-gatherers might have simply chased and eaten stray animals. But they think it's more likely that the fisher-folk decided there was a better way to bring home the bacon.

—MICHAEL BALTER



Spotted! Remains of domesticated pigs that resembled this Bentheimer breed were found in settlements of ancient hunter-gatherers

10,000 years ago in the Near East, although farmers in Europe later domesticated the wild boar that roamed that continent.)

To tell whether the pig bones were from domestic or wild animals, the team studied classical markers such as the sizes of bones and teeth. They also gleaned clues



A Teacher's Quest

Biologist Steve Robinson is leaving a high-level White House post for a high school in New York City in a career-long quest to understand the secret behind great teaching

SOME SCIENTISTS COME TO WASHINGTON and contract Potomac fever—a disease triggered by the proximity to power that produces a hunger for national attention. And then there's Steve Robinson.

What motivates the former university biology professor turned secondary school science teacher turned education policy-maker isn't power. It is figuring out what it takes to be a good teacher. He has looked for answers at every stage of his unusual career arc, both to improve his own skills and to contribute to the ongoing debate about how to boost the quality of the nation's teacher corps. And this week, at the age of 62, Robinson begins his latest quest.

Last month, Robinson walked away from an influential education policy position at the White House to teach science to disadvantaged students attending Democracy Prep Charter High School in New York City's Harlem neighborhood. (Charter schools are run by private organizations but receive govern-

ment money to teach students who would otherwise attend public schools.) He hopes to make use of what he's learned in Washington and as a teacher at both the secondary and college level to hit an educational trifecta: Prepare his students for college and career success, make himself into a better teacher, and foster Democracy Prep's role as a test-bed for improving U.S. K–12 education.

"There are lots of things I could have done," Robinson says about his decision. "But I wanted to teach. I want to see whether I'm a good teacher."

Those who know him are not surprised by his latest career twist. "Steve is a person who would follow his star," says Edward Klekowski, a retired plant biologist at the University of Massachusetts (UMass), Amherst, who 25 years ago was Robinson's departmental colleague and running partner. "He was always an idealist. And it sounds like he still is."

Robinson's biggest challenge will be

Gearing up. Steve Robinson (*center*) trains with Democracy Prep assistant principal Brian Martin and fellow physics teacher Dipti Dedhia.

applying his policy experience to the day-to-day problems of teaching in an urban school, says another friend, George Sugai, an education professor now at the University of Connecticut, Storrs, who taught Robinson while he was earning a master's degree in 2002 at the University of Oregon. "It's the handshake—how to transfer his knowledge of what works to these kids," Sugai says. "But I don't think it'll take him long."

Be like Benton

Growing up in a wealthy suburb of Chicago, Illinois, Robinson says that he never envisioned working at the White House. But he did think a lot about becoming a science teacher. In fact, last year Robinson recorded a public tribute to his former high school biology teacher (StoryCorps.net).

"Mr. Benton, I always wanted to grow up and be you," Robinson told James Benton, now 81, who taught for 37 years at Lake Forest High School before retiring in 1994. In addition to thanking Benton "for making science fun," Robinson praises his Socratic approach to teaching. "You would often answer my question by saying, 'Well, think about it.' Perhaps it was your way of saying that science is not a group of facts, but rather a different way of thinking about things."

Benton's influence helped steer Robinson toward an undergraduate degree from Princeton University and a Ph.D. in cell and molecular biology at the University of Michigan. "People said I was pretty good at research, so I stuck with it," says Robinson in a matter-of-fact tone that reflects his no-frills personality.

In 1984, he landed a tenure-track position in the botany department at UMass.

But once there, he realized that being a successful academic required more than a love of bench science. "I didn't like running a small lab and scrounging for research funding."

Robinson says that he chose not to go up for tenure because he couldn't imagine spending his entire career as an academic. Former colleagues say that his modest research productivity would have made it extremely difficult for Robinson to meet the university's benchmark. At the same time, however, his classroom skills generated rave reviews.

"He was one of those gifted teachers who could explain something complex really clearly," says Klekowski, who taught a seminar with Robinson and sat in on one of his courses. "He was on the cutting edge of plant molecular biology, and there should have been a place for him at the university as a distinguished teaching professor. But there wasn't."

Once Robinson decided to leave academia, he headed west and eventually settled in Eugene, Oregon. (His wife, Jan Cuny, was a member of the computer science department at UMass, and in 1993 she joined the University of Oregon faculty.) Robinson worked in a university lab and helped raise the couple's three children. But soon the itch to teach returned.

He tried to scratch it by spending 5 years as the only science teacher at a small private school. But it wasn't quite what he wanted. "I'd always been a public school person," he says. "It's one of the great American innovations."

Public school teachers need to be certified by the state, however, and for Robinson that meant going back to graduate school for the additional course work he needed to become eligible. But the classes and student teaching brought him no closer to his goal of "becoming a more effective teacher, whatever that means." Nor did the next 3 years he spent at North Eugene High School.

Robinson believes that many teachers fail to convey the essence of how science is actually done. "Everybody teaches the scientific method—developing a hypothesis and then doing the experiments to test it," he says. "But scientists don't actually follow the scientific method." Instead, Robinson's academic training has taught him the importance of the right hypothesis in making progress. "Most of the time when you do an experiment, it says to you, 'Hey buddy,

that wasn't the right experiment.' You didn't ask the question well enough, or there's something that you just didn't think about."

Of course, knowing the subject matter is also important, and he says teachers who lack that knowledge can do serious harm. "I would even go so far as to say that many of my students would be better off if they never had a science teacher before me because they have to unlearn so many bad things" from elementary school teachers who were afraid of science.

Robinson is confident that he understands what he is trying to teach: "It's pretty hard to stump me on content," he says. But he was disappointed in the lack of pedagogical guidance that he received during his high school teaching career. "Three times a year I'd be asked when I wanted to be evaluated, and I'd say, 'Why don't you come next Wednesday?' You'd have to be a total idiot not to have three good days of lessons in your back pocket. And the other 177 days I could do anything I wanted."

The follow-up was no more rigorous, he adds. "There'd be a checklist, with a scale of 1 to 5. I'd get mostly 5s, and a few 4s. When I asked about the 4s, they'd say, 'Well, there's always room for improvement, right?'"

Obamaworld

In 2004, Cuny, his wife, decided to come work at the National Science Foundation to start a program on broadening participation in computer science. Looking for a way to

join her in Washington, Robinson applied for and won a federally funded fellowship allowing classroom teachers to work for Congress or a federal agency. In the fall of 2005, he lucked into a job with the newly elected Democratic senator from Illinois, Barack Obama. "They were looking for someone who knew something about education, and I was free," Robinson says.

Within 2 weeks, Robinson was helping to write a speech in which Obama previewed many of the policies that have marked his administration's approach to education. They include incentives for school districts to raise standards, linking teacher evaluations to student performance, and creating better tools to assess learning. He also emphasized the importance of a well-trained teacher workforce, including the use of alternative certification, a popular tool for charter schools to attract people who might not otherwise consider teaching.

Those ideas resonated with Robinson, who quickly fell in love with the job. "I thought I would be here a year," he says. "But [Obama's] office was a great place to work. There was an amazing group of talented people. If I had been in another office I probably would have returned [after the fellowship ended] to Oregon."

Robinson rose swiftly in the Washington policy world. After the 2008 election, he became special adviser on STEM (science, technology, engineering, and mathematics) education to Education Secretary Arne Duncan. In September 2009, he became special assistant on the White House Domestic Policy Council, where STEM education was part of his portfolio.

That position—"I had the best policy job in Washington"—gave him a front-row seat in the political tug-of-war with Congress over revising the Bush administration's unpopular No Child Left Behind Act, the 2002 law that spells out the federal government's role in elementary and secondary education. He also worked closely with officials from a dozen federal agencies to develop a strategic plan to improve the government's \$3 billion investment in STEM education.

Despite his loyalty to Obama and his policies, Robinson was upset earlier this year when the administration proposed to reshuffle the federal STEM portfolio by designating three lead agencies and drastically cutting back the involvement of other agencies (*Science*, 26 July, p. 338). "I'll be honest," he says. "It's hard for me to defend [that proposal] because I argued against it pretty strongly." But he emphasizes that the budget proposal "is not the reason I'm leav-

"Sure, working at the White House means long hours. But teaching is just plain exhausting."

ing. I want to make that really clear.”

Robinson says that he definitely wasn't looking for a new job last September when he heard Seth Andrew, a former teacher and the founder of Democracy Prep, talk about improving K–12 education at an event that included Duncan and other education luminaries. But what he heard that day at the Brookings Institution rekindled his passion for the classroom.

Charter schools are a rapidly growing part of the U.S. education landscape. Their flexibility pleases many conservatives, who want to reduce the federal role in education. But their autonomy worries some liberals, who fear that the charter movement threatens a 200-year tradition of public education.

tices, a culture of high expectations, and high-quality teaching. Their approach to teacher development has a special allure for Robinson. “People think it’s just about setting high standards and firing bad teachers,” he says. “But it’s really about how to cultivate good teaching.”

Invited to deliver a 40-minute practice lesson to students as part of the school’s hiring process, Robinson was impressed by the extensive feedback that he received from staff and school administrators. “They gave me a very thorough debriefing of what I had done well and poorly,” he says. “They asked me how I would have taught the class differently if I had to do it again tomorrow and next week. Nobody had ever asked me that

at Democracy Prep will also promote one of the original goals of the charter school movement. “The promise of charter schools was that they would be laboratories for innovation that would help us improve public schools,” he says. “But we’ve failed to do that. Instead, we’ve allowed an amazing variability in the system—many schools are terrific, and some are horrible.

The solution, according to Robinson and many educators, lies in improving the quality of the professional workforce. And despite his admiration for his former high school teacher, Robinson takes issue with Benton’s assertion during last year’s recorded conversation that “a good teacher is born, not made, and that most teaching is intuitive.” Likewise, Robinson says, studies that show a teacher with a master’s degree is no more effective in the classroom than someone with an undergraduate degree are flawed.

“First of all, a master’s degree is a very large variable,” Robinson says. “You can get one off a matchbook cover, or you can get one in math education from Deborah Ball [dean of education at the University of Michigan]. And you can’t convince me that studying with Deborah Ball for a year doesn’t make you a better teacher.

“As educators, we have to believe that making people smarter can help. So if the studies don’t show an effect, all it means is that we’re not doing a good job of teaching those people what they need to know.”

The poor quality of most teacher professional development—improving the skills of those already in the classroom—aggravates the problem, he believes. “As a teacher, if I said I was going to teach all my students the same way, it would be considered professional malpractice,” Robinson says. “But that’s what we do with most professional development.

“We assume they all need the same thing. And that’s why almost no professional development works. It needs to be differentiated, but to do that, you need a good evaluation system. And that’s been a hard political battle.”

Robinson acknowledges that he is taking a big risk by returning to the classroom after 8 years in the political arena. But he says that it feels like the right thing to do. In the meantime, he scoffs at former colleagues who teased him about deciding to take it easy. “Sure, working at the White House means long hours,” he says. “But teaching is just plain exhausting.” And while he may be under less scrutiny in Harlem than in the White House, Robinson hopes that his work will have just as big an impact on U.S. education.

—JEFFREY MERVIS



Role model. Steve Robinson thanks his high school biology teacher in a StoryCorps event at the White House.

Democracy Prep, which began in 2006 and now operates eight schools in Harlem and one in Camden, New Jersey, is part of a wave of so-called no excuse charters that has attracted national attention by sharply raising test scores among traditionally low-achieving populations. “One of their schools is the highest performing charter in New York City,” Robinson notes. The organization is also growing rapidly: Last year, it received a 5-year, \$9.1 million federal grant to train the additional staff members needed to open or expand another 15 schools in New York City and elsewhere.

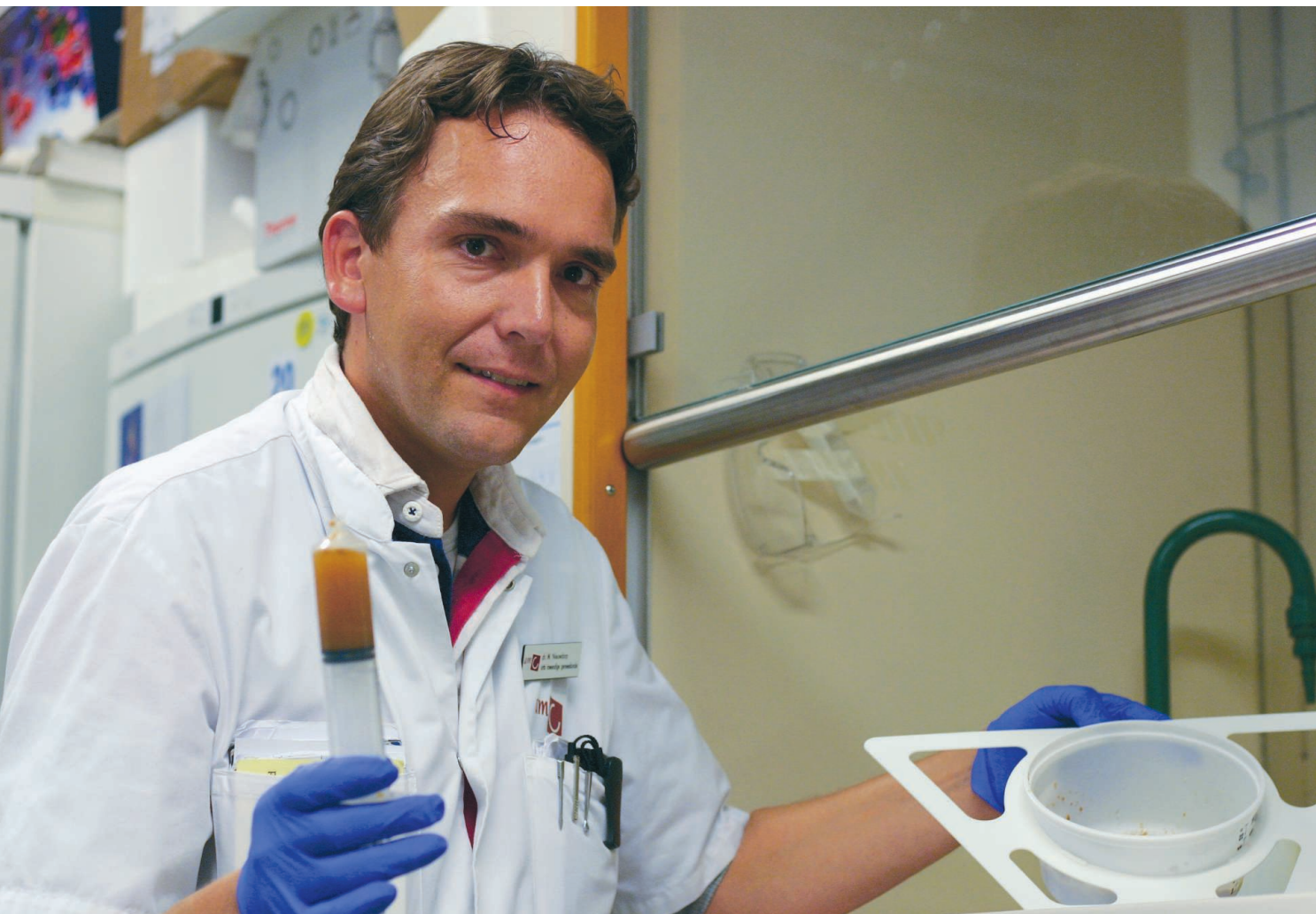
School officials say that their success is based on a five-part formula—more time in the classroom, targeted interventions for students who need them, an extensive use of student performance data to improve prac-

question. One of the things that schools like Democracy Prep do well is to create a culture in which teachers support each other and offer constructive criticism.”

Katie Duffy, who succeeded Andrew this year as the organization’s CEO, says that culture is critical to what the school hopes to achieve. “It isn’t enough to come in as a good teacher,” says Duffy, who has been with the school since its early days. “We want to be sure they continue to get better.” The absence of tenure for teachers may also play a role.

Robinson says the school’s emphasis on civic engagement was another attraction. “That civic mission appealed to me because of all the time I’ve spent in Obamaworld,” he says. “I also think that teaching in Harlem is very much of a public act.”

In fact, Robinson hopes that working



MEDICAL RESEARCH

The Promise of Poop

Fecal transplants offer hope for treating many diseases. But they need to be studied more scientifically, says one of the treatment's pioneers

AMSTERDAM—Soon after Max Nieuwdorp started his residency at the internal medicine department of the Academic Medical Center (AMC) here in 2006, he was confronted with a sad case: an 81-year-old woman hospitalized for a complication after a urinary tract infection who seemed unlikely to survive. She had bed sores and high fevers and was unable to eat. After antibiotics had wiped out her colon's microbial population, an opportunistic bacterium called *Clostridium difficile* had taken over, causing terrible diarrhea and bowel inflammation.

C. difficile is a notorious pathogen that kills at least 14,000 patients a year in the United States alone; many patients suffer repeated bouts with the microbe. To treat

it, the woman was given several courses of vancomycin, the standard antibiotic in such cases. But, as often happens, the bacteria had become resistant.

Nieuwdorp refused to accept the patient's fate—"I was young and naive," he says—and started searching PubMed for anything that could save her. When he found a 1958 paper by Ben Eiseman, a physician who was then at the University of Colorado, Denver, he knew what to do. "I want to try a fecal transplant," he told his supervisor, Joep Bartelsman.

Once he realized that Nieuwdorp wasn't joking, Bartelsman agreed. The plan was simple: The duo would flush the contents from the woman's colon, including, hope-

fully, the *C. difficile* population, and replace it with the healthy bacterial flora from a donor, in this case her son. To do so, they would mix the son's feces with saline in a blender and squirt it straight into the patient's duodenum, the upper part of her intestine, via a thin plastic tube inserted through her nose.

Three days after her treatment, the woman left the hospital—walking. Nieuwdorp and Bartelsman decided to treat another six *C. difficile* patients in the following months. Embarrassed about the unusual experiment, they waited for colleagues to break for lunch before infusing the stools. Four patients recovered immediately, the other two after another transplant from a second donor. The transplanted bacteria were apparently restoring the intestinal flora to health.

But when Nieuwdorp presented the results at a hospital meeting, an internist approached him with a condescending smile. "If you seriously want us to treat our *C. diff* patients with poop, why don't you infuse our cardiovascular patients as well?" the man asked, and left the room.

Golden brown. Max Nieuwdorp prepares a human stool for transplantation.

That skepticism is gone. Many doctors now agree that intestinal *C. difficile* infections can be cured by transplanting stools from healthy people. Backed by a growing body of work linking the gut's microbial ecosystem to overall health, researchers also think that a wholesale replacement of the gut's microbial flora might help treat many other diseases, such as inflammatory bowel disease, diabetes, and the elusive chronic fatigue syndrome. More and more doctors perform fecal transplants, and online manuals for patients desperate enough to overcome the yuck factor show how to do it yourself.

What's still missing is a truly scientific approach to fecal transplants, says Nieuwdorp, who has become a leading advocate of more research. A paper that the AMC group published in *The New England Journal of Medicine* (NEJM) in January described a randomized controlled clinical trial of the transplants—the first such study ever reported. Nieuwdorp has also set up collaborations with lab scientists to better understand the underlying mechanisms. He hopes that these studies will eventually allow doctors to move from stool transplants to a more subtle approach: administering selected bacterial strains.

Becoming mainstream

The pioneering Eiseman paper, published in *Surgery*, described how the anal infusion of liquidized stool cured four patients from a disease called pseudomembranous enterocolitis, which had symptoms very similar to a severe *C. difficile* infection, although it was likely caused by a different microbe. It wasn't the first medical use of poop; fecal suspensions to treat food poisoning and severe diarrhea were first reported in the 4th century by a Chinese doctor and writer named Ge Hong, and as early as the 17th century, they were used to treat cows with intestinal problems.

Eiseman's paper was followed by occasional case reports, but for decades, the medical community paid little attention. Antibiotics had rendered the primitive, distasteful technique obsolete, it seemed.

Nieuwdorp didn't initially put his money on it either. After his first seven patients, he went to the University of California, San Diego, as a postdoc to study sugar molecules on the linings of blood vessels and intestines. After his return to Amsterdam in 2008, he resumed his work on the relation between gut microbiota and metabolism. He



GUT FEELING

Researchers have reported positive effects with fecal transplants in more than 15 different diseases, but for most, the evidence is still weak.

GASTROINTESTINAL DISEASES

- Recurrent infection with *C. difficile* (photo above) 4
- Irritable bowel syndrome 3
- Chronic constipation 3
- Ulcerative colitis 3
- Crohn's disease 3

NONGASTROINTESTINAL DISEASES

- Metabolic syndrome 4
- Chronic fatigue syndrome 2
- Multiple sclerosis 2
- Idiopathic thrombocytopenic purpura 2
- Autism 2
- Parkinson's disease 1
- Rheumatoid arthritis 1
- Sacroiliitis 1
- Halitosis 1
- Acne 1
- Insomnia 1
- Depression 1

KEY:

- 4 Randomized, controlled trial
- 3 Case series published
- 2 Isolated case(s) published
- 1 Unpublished clinical observations

chose a Dutch medical journal to publish his fecal transplant results in that same year.

But recurrent *C. difficile* infections were still on the rise, as was antibiotic resistance in the bacteria. Interest in fecal transplants grew in the United States after a 2010 article in *The New York Times* about the successful treatment of a very serious *C. difficile* case by Alexander Khoruts, a gastroenterologist at the University of Minnesota Medical Center in Minneapolis. "I realized that to let this therapy become accepted by the community of physicians, we would have to do a randomized clinical trial," Nieuwdorp says.

That study compared fecal transplants with vancomycin, the standard treatment for *C. difficile*, or vancomycin combined with bowel flushing. The researchers aimed to enroll 120 patients, but the study's data and safety monitoring board halted the study after just 43 patients, because continuing would be unethical: Ninety-four percent of the transplant patients were cured, versus 31% and 23%, respectively, in the control groups. The resulting *NEJM* paper "did bring the procedure closer to mainstream medicine," Khoruts says.

More evidence needed

Some doctors needed no convincing. One was gastroenterologist Thomas Borody of the Australian Centre for Digestive Diseases in Five Dock, who since 1988 has performed fecal transplants in more than 3000 patients, suffering not just from *C. difficile* but also from irritable bowel syndrome; inflammatory bowel syndrome; constipation; arthritis; and sacroiliitis, an inflammation of the sacroiliac joint. Borody has published some of his results; last year, for instance, he reported some improvement in 92% of 62 ulcerative colitis patients treated with fecal transplants and full recovery in 68%.

But although Borody is widely recognized as a pioneer, he has never carried out a randomized trial. Other researchers have reported encouraging results for non-gastrointestinal disorders that appear to be associated with changes in the microbial flora, such as Parkinson's, autism, and multiple sclerosis. In most cases, however, the claims are based on a few cases, or a series of cases without a control group. (See table, left.)

More rigorous trials are now under way, including one in ulcerative colitis at McMaster University in Hamilton, Canada, and another in Crohn's disease at Nanjing Medical University in China. Nieuwdorp himself has just embarked on his second trial in patients with metabolic syndrome, the dysregulation of the body's metabolism as a result of overweight that is often a precursor to diabetes. Last year, his group reported that transferring the intestinal microbiota from lean donors increases insulin sensitivity in these patients—an encouraging sign.

Meanwhile, Lawrence Brandt of the Montefiore Medical Center in New York City, another fecal transplant pioneer, is doing a second trial with recurrent *C. difficile*. The study started before Nieuwdorp's *NEJM* paper, and unlike Nieuwdorp's, it is blinded and includes a placebo group. Both the patient and a donor donate their stool; the patient is infused with either the donor

Regulators Grapple With an Unorthodox Therapy

Taking one person's stool and putting it into the gut of another may be simple enough—but regulating it is not. The rapid rise of fecal transplantation, coupled with pressure from enthusiastic patients and doctors, has regulators wondering how to respond. The procedure occupies an odd place somewhere between tissue transplants and “probiotic” therapies—infusions of beneficial bacteria—and its risks are unknown. Coming up with sensible regulation is even harder because unlike, say, a blood transfusion, anyone can perform a stool transplant at home.

So far, the European Medicines Agency has not stepped in to regulate fecal transplants; the only thing that doctors need to do is get the patient's informed consent and comply with a donor screening protocol. Australia, where gastroenterologist Thomas Borody has carried out thousands of fecal transplants (see main story, p. 954), has similarly relaxed regulations.

In the United States, by contrast, the Food and Drug Administration (FDA) recently asserted its authority over the procedure. At a workshop on 2 and 3 May in Bethesda, Maryland, FDA's Jay Slater said that transplants are considered “an unapproved new drug.” That meant any transplant can be done only as part of an official clinical trial, and doctors need to file an Investigational New Drug (IND) application—which can take months. (The agency said it might make exceptions for life-threatening conditions on a case-by-case basis.)

An FDA spokesperson says that Slater's comments didn't represent a policy change. But many researchers interpreted them as a clampdown. Lawrence Brandt of the Montefiore Medical Center in New York City says that he rejected 25 patients suffering from recurrent *Clostridium difficile*

infection—where the evidence that fecal transplants work is strongest—after hospital lawyers told him to stop.

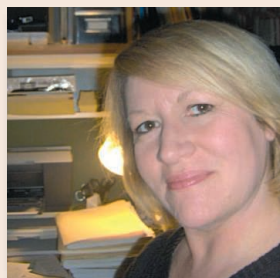
Patients and doctors have long urged the agency not to interfere with the treatment. At the Bethesda workshop, FDA officials heard testimony from Catherine Duff, a 57-year-old former medical case manager from Carmel, Indiana, who related how a fecal transplant had cured her recurrent *C. difficile* infection—and saved her life. “Please, do something not only for me, but for all those around the country and everywhere,” she implored the FDA. “Please do something quickly.”

Duff's appearance “definitely left an impression on the FDA officials,” says Alexander Khoruts, a gastroenterologist at the University of Minnesota Medical Center in Minneapolis who attended the meeting. In new guidelines issued on 18 July, the agency announced it would “exercise enforcement discretion” in the case of *C. difficile*—meaning that, if they meet certain conditions, doctors can perform fecal transplants without prior approval. An IND is still needed for all other diseases and for *C. difficile* therapy in infants. The new guidance is widely regarded as a stopgap measure that gives the agency time to develop more comprehensive regulations.

FDA's task might get easier if scientists can replace fecal transplants with standardized mixes of microbial strains grown in the lab, which are simpler and presumably less risky. On 29 July, FDA approved a phase II trial with such a cocktail, developed by a company called Rebiotix. But Canada's federal regulatory agency clamped down on a similar product. After an academic collaboration named RePOOPulate treated two *C. difficile* patients with a mixture of 33 stool-derived microbial strains, Health Canada ordered a halt to the procedure in 2011; it said the mix is a “synthetic” product for which drug approval is needed and gave the scientists a long list of criteria to meet.

“Actually, they are not unreasonable,” says RePOOPulate project leader Elaine Petrof. “Health Canada realizes this technique is not going away, and they want to sort this out with us.”

—JDV



Patient's voice. Catherine Duff spoke up at an FDA workshop in May.

stool or his own. That design is now standard, says Nieuwdorp, who uses it in the metabolic syndrome trial as well. “Some people claimed to smell that they had not received their own poop,” he says. “They weren't always right, actually.”

Borody says he is “very much for blinded trials”—he is involved in one with ulcerative colitis himself that is about to start at his university. But in the case of *C. difficile*, Nieuwdorp's study wasn't needed, he says—let alone Brandt's—because the evidence from case series was already overwhelming. “This trial should not have been approved by the ethics committee in the first place,” Borody says, comparing Nieuwdorp's study to a randomized controlled trial to test whether parachutes can save lives.

Begging for a transplant

As word of mouth about fecal transplants has spread, many other doctors have gotten in on the game, and many private clinics see handsome profits from a relatively

simple procedure. “The first generation of physicians involved in this technique were of the most idealistic type I can imagine,” Khoruts says. “This changed, unfortunately.” Patients are often desperate for the treatment; Nieuwdorp says he receives frequent phone calls, e-mails, and visits from people begging him for a transplant. He says that he rejects all such requests but refers some people to Borody.

Meanwhile, do-it-yourself transplants appear to be on the rise as well. Instructions on how to flush out your colon, prepare donor stool—at “chocolate milkshake thickness,” as one website helpfully explains—and infuse it by enema or nasal tube have proliferated on the Internet. In one YouTube video, a patient describes how putting Vicks VapoRub under one's nose can help suppress the odor. There have even been reports about people drinking liquidized feces.

Such experiments are worrisome, scien-

tists say, because fecal transplants aren't without risks. Earlier this month, for instance, two patients were reported to have developed norovirus gastroenteritis and diarrhea around the time of their fecal transplant. (The donors had tested negative for norovirus, and the doctors believe the patients may have picked up the virus somewhere else.) Also this month

came a report of a 78-year-old man whose ulcerative colitis flared up instead of disappearing after a fecal transplant—although that may have been because his doctors stopped administering prednisone, a drug that reduces inflammation, prior to treatment.

To reduce the risks, most doctors screen donors for a battery of harmful viruses, bacteria, and parasites that might be transmitted through stool—including HIV, hepatitis B and C viruses, cytomegalovirus, Epstein-Barr virus, *Campylobacter jejuni*, and *Blastocystis*. But they can't be sure they're catching everything, and noninfectious dis-

Online

sciencemag.org

Podcast interview with author Jop de Vrieze (http://scim.ag/pod_6149).

eases are a concern as well. Diabetes, atherosclerosis, autism, and colorectal cancer have all been shown to be associated with certain gut microbiota compositions. Scientists are only beginning to tease out cause and effect, but Khoruts says that it's best to err on the side of caution and not use donors whose gut flora might cause trouble.

Khoruts is investigating the optimal donor characteristics and has set up a donor bank with frozen stool from healthy people who meet a long list of criteria. So far, most of his patients have brought in family members as donors, and few of them are in perfect health. "If the patient is 70 years old, you won't need a Greek god to cure her," Khoruts says. "But for a younger patient, you give bugs for the rest of his life—you want something better."

Underlying mechanisms

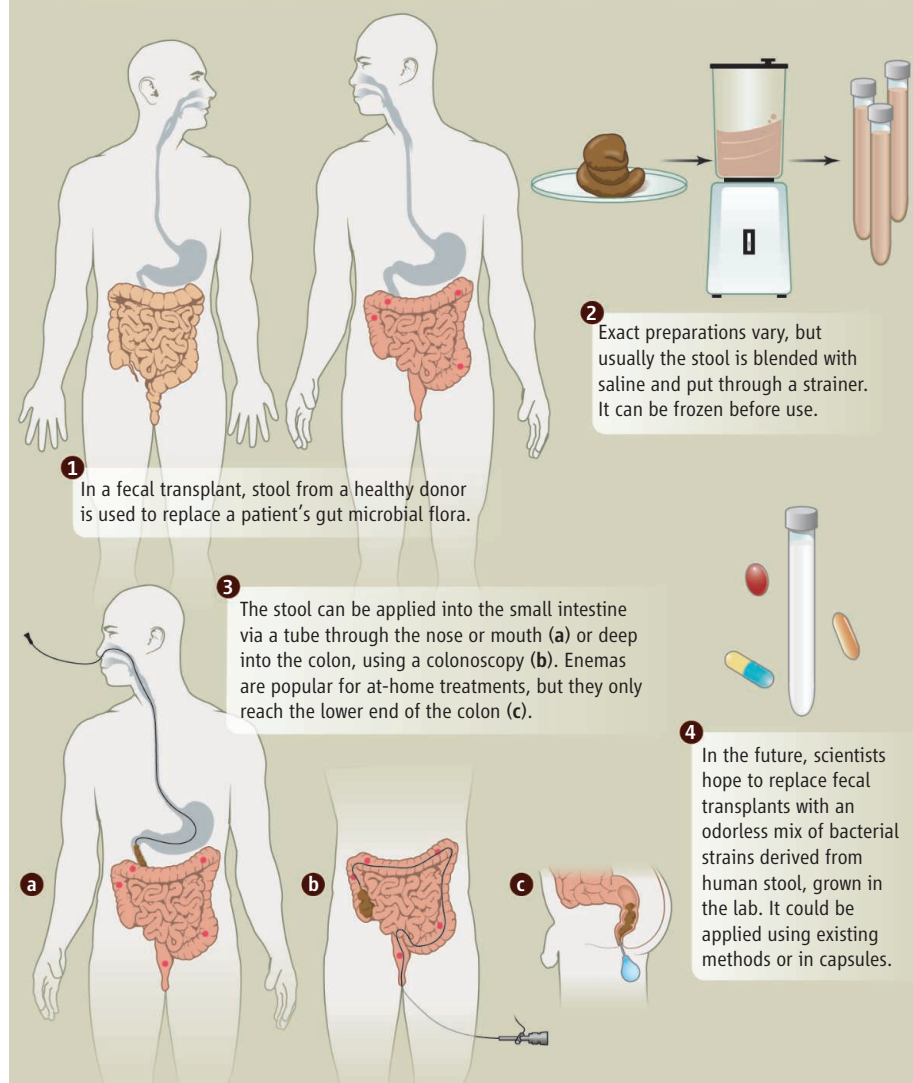
Knowing how fecal transplants work is key to making them safer. Do the donor populations take up residence in the gut after the transplant? Which strains make the difference, and how do the transplanted microbes interact with the resident ones? Nieuwdorp collaborates with Willem de Vos, a microbial ecologist at Wageningen University in the Netherlands, whose expertise is anaerobic bacteria, the group that dominates in the human gut. "We have shown that in *C. difficile* patients, some important species are absent, while others that you don't want are dominant," De Vos says. His research has also shown that the low microbial diversity in *C. difficile* patients is comparable to that of a 1-year-old child. But after a transplant, anaerobic bacteria from the donor settle in the recipient's gut and the diversity is restored.

Nieuwdorp also works with Fredrik Bäckhed of the University of Gothenburg in Sweden, who runs a facility housing mice that grow up without a single bacterium in their bodies. This allows the scientists to investigate the effects of specific microbial strains. "We are playing with different donors to find the superbacteria that make the difference between health and disease," Nieuwdorp says.

The hope is that in the end, doctors can abandon the poop and infuse just these bacteria. Such cultured cocktails might have downsides, however. They could be less powerful than the complete ecosystems found in stool, Borody says, and the bacteria might mutate in the lab, losing their healing power, as they are grown generation after generation.

Still, many others believe the cocktails are the way to go. A group led by Kenya Honda at the University of Tokyo recently reported curing mice of colitis and allergic

HOW FECAL TRANSPLANTATION WORKS



diarrhea by treating them with 17 harmless *Clostridium* strains that had previously been shown to induce regulatory T cells of the immune system, quenching an overactive immune response.

In a project called RePOOPulate, a Canadian team led by Elaine Petrof of Queen's University in Kingston and Emma Allen-Vercoe from the University of Guelph has developed a stool-derived set of 33 microbial strains for the treatment of *C. difficile* and inflammatory bowel disease. They hope the strains will offer the benefits of a full fecal transplant but with less risk. Allen-Vercoe initially cultured 70 strains, from which Petrof made a selection based on each strain's pathogenicity and resistance to antibiotics. For the final selection, she says she used her judgment: "Would I put this bug into my mom? No? Then I would take it out."

A U.S. company called Rebiotix is going

the same route; the Food and Drug Administration recently greenlighted a phase II clinical trial of its mix of several hundred stool-derived strains to target *C. difficile* infection. "We don't consider our product a fecal transplant," Rebiotix founder and CEO Lee Jones writes in an e-mail. "Instead, we are developing a microbiota restoration therapy in the form of a biologic drug."

Nieuwdorp sees enormous possibilities for such therapies—but he says it will take time. "I'm 36 now. I'll be happy if by the time I'm 60, microbiota analysis will be standard procedure in hospital labs," he says. For now, he's happy that the taboo on fecal transplants is gone. At his own center, "specialists are lining up to test the impact of transplant on 'their' diseases," Nieuwdorp says. And yes, the list now includes cardiovascular diseases.

—JOP DE VRIEZE

Jop de Vrieze is a science writer in Amsterdam.

LETTERS

edited by Jennifer Sills

Evidence-Based Environmental Laws for China

IN THE POLICY FORUM "REVISING CHINA'S ENVIRONMENTAL LAW" (12 JULY, P. 133), G. HE *ET AL.* discuss the need for a sound legal and scientific basis for such a revision. They regard unclear punishment rules as a setback. We are concerned that even clear punishment will serve as a setback, if that punishment ignores scientific evidence and gives the wrong impression of how to effectively preserve habitats and biodiversity.

*Teinopalpus aureus.*

better understood (3), making captive breeding possible. Even without these new discoveries, it does not make sense to list insects in the same category as mammals because of their fundamentally different population dynamics.

In addition to disregarding current scientific knowledge, such extreme punishments imply that conservation of insects can be achieved simply by protecting them from collectors, whereas the main problem is the vast and rapid destruction of habitats (4). We hope that future environmental laws in China and elsewhere will reflect this difference.

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Call for Prudence in Whole-Genome Testing

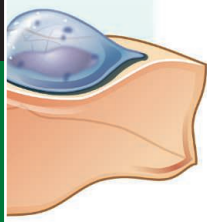
IN THEIR POLICY FORUM "PATIENT AUTONOMY and incidental findings in clinical genomics" (31 May, p. 1049; published online 16 May), S. M. Wolf *et al.* criticize the American College of Medical Genetics and Genomics (ACMG) for giving up on well-established principles of patient autonomy and informed consent. Like Wolf *et al.*, the European Society of Human Genetics (ESHG) recently called for more prudence (1).

The ACMG presents diagnostic testing based on whole-genome sequencing as an opportunity for screening. The ESHG position is more narrow: Whenever possible, such testing should be targeted to genome regions linked to the patient's indications. Wider testing requires justification in terms of necessity. Adding screening targets to a diagnostic test violates the necessity criterion. Imposing this extra testing on patients who need an answer to their clinical problem is at odds with respect for autonomy. As stated by Wolf *et al.*, people have a right to decline testing on the basis of their own assessment of burdens and benefits.

With regard to reporting unsolicited findings, the ESHG does acknowledge that the patient's "right not to know" may sometimes have to give way to professional responsibilities. The patient may not have foreseen and considered a specific finding, and in some cases the physician has a moral duty to warn the patient's close relatives. Where testing of children is concerned, the child's best interest may supersede the parental "right (not) to know" (2). Pending further debate, we urge a more cautious attitude specifically including the patients' and physicians' perspectives.

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Tension on the
nucleus

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IBI Prize Essay

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Data Disclosure Crucial After DNA Patent Verdict

THE SUPREME COURT'S RECENT DECISION in *Association for Molecular Pathology v. Myriad Genetics* (1) was widely regarded for its conclusion that patents could not be held over isolated DNA. However, Myriad maintains a confidential library of BRCA variants of undetermined significance (VUSs) (2).



These are changes in DNA that have not yet been characterized as either benign or pathogenic and are found in about 7% of women who have their BRCA genes tested (3). Over the 12 years that it held an effective monopoly over such testing in the United States, Myriad developed a VUS database that correlates particular sequences with the clinical history of both the patient who was tested and her family.

Perhaps in anticipation of its BRCA patents' expiration, Myriad stopped publishing the information in its VUS database some years ago. In fact, Myriad last provided data to the federal Breast Cancer Information Core database in 2004 (4). Two years later,

a group that included Myriad scientists published a paper listing 118 more mutations (5). But in the past 7 years, Myriad has kept the database containing the information on VUS unequivocally to itself. That means that Myriad (and its overseas licensees) can now justifiably claim to be in a better position to assess the clinical significance of BRCA results than any of their competitors.

The Myriad case therefore demonstrates how the provider of a diagnostic test can acquire a decisive competitive advantage simply through a market idiosyncrasy. The existence of such pseudo-proprietorship does not only have implications for clinical diagnosis and therapy; it also has the potential to forestall research. Two policy changes within the United States may address these problems (6). The first involves the imposi-

tion of disclosure requirements on genetic testing laboratories as a precondition for the grant of their certification. Organizations such as the Centers for Medicare and Medicaid Services in the United States and the European Society of Human Genetics in Europe have been identified as suitable agencies of those tasks. The second policy change entails the imposition of prospective disclosure requirements as one of the criteria for national registration of the test. Such regulation in the United States could most effectively be overseen by the Food and Drug Administration in conjunction with the National Institutes of Health.

ALEX DE COSTA

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CORRECTIONS AND CLARIFICATIONS

News & Analysis: "Fragile wetland will test Turkey's resolve in protecting biodiversity" by J. Bohannon (26 July, p. 332). In the final paragraph, State Hydraulic Works, not the Ministry of Development, should be named as the granter of dam contracts. The HTML and PDF versions online have been corrected.

News of the Week: "Cancer knife sniffs out cancer cells" (19 July, p. 221). Jeremy Nicholson was incorrectly identified as a surgeon. He is a biochemist and head of the Department of Surgery and Cancer at Imperial College London, UK. The HTML and PDF versions online have been corrected.

Letters: "Turkey must end violent response to protests" by E. Altindis *et al.* (19 July, p. 236). The Letter indicated that "[m]ore than 4000 academics around the world have already signed a petition to protest the police brutality" and provided a link to the petition at <http://academicsforgezi.com/our-call/>. The link referenced the English version of the petition, which had amassed over 2200 signatures at the time of publication. The Turkish version of the petition (<http://academicsforgezi.com/>), which was in the original Letter draft, had accrued more than 1800 additional signatures. The English and Turkish petitions together made up the 4000 signatures cited by the authors. Reference 14 should have included both URLs to clarify the sources of the signatures. The reference has been corrected in the HTML and PDF versions online.

Reports: "Loss of function of the melanocortin 2 receptor accessory protein 2 is associated with mammalian obesity" by M. Asai *et al.* (19 July, p. 275). In the first sentence of the eighth paragraph, the word "loss" was omitted from the final phrase. The phrase should be "depending on the degree to which Mrap2 loss interferes with Mc4r function." The HTML and PDF versions online have been corrected.

Reports: "Infectivity, transmission, and pathology of human-isolated H7N9 influenza virus in ferrets and pigs" by H. Zhu *et al.* (12 July, p. 183, Published Online 23 May). In the Acknowledgments, the GenBank accession numbers were incorrectly reported. The correct numbers are KF021594 to KF021601. The HTML and PDF versions online have been corrected.

Reports: "LRP6 mutation in a family with early coronary disease and metabolic risk factors" by A. Mani *et al.* (2 March 2007, p. 1278). In Fig. 3, the label for the x axis should have been "Wnt 3a (ng/ml)," not "Wnt 3a (pg/ml).

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

PHYSICS

Rebirthing Pains

Huw Price

Physicist Lee Smolin likes big targets. His previous book (*I*) took on the string theorists who dominate so much of contemporary theoretical physics. A few years ago, it was my engrossing in-flight reading on a trip to the Perimeter Institute, where I first met its rather engaging author. I thoroughly enjoyed that battle, from my distant philosophical vantage point—“Pleasant is it to behold great encounters of warfare arrayed over the plains, with no part of yours in the peril,” as Lucretius noted (2). But now things are more serious: in *Time Reborn* Smolin has my team in his sights, and some part of mine is certainly in the peril, if he emerges victorious. Should I now be feeling sorry for the string theorists?

I’ll come back to that question, but first to the dispute itself, which is one of philosophy’s oldest feuds. One team thinks of time as we seem to experience it, a locus of flow and change, centered on the present moment—“All is flux,” as Heraclitus asserted around 500 BCE. The other, my clan, is loyal instead to Heraclitus’s near contemporary, Parmenides of Elea. We think of time as it is described in history: simply a series or block of events, lined up in a particular order, with no distinguished present moment. For us, now is like here—it marks where we ourselves happen to stand but has no significance at all from the universe’s point of view.

Which side is right? Both teams have supporters in contemporary philosophy, but we Parmenideans claim powerful allies in modern physics, commonly held by physicists themselves to favor the block picture. Einstein is often quoted as one of our champions. In a letter to the bereaved family of his friend Michele Besso, Einstein offered the consoling thought that past, present, and future are all equally real—only from our human perspective does the past seem lost: We physicists “know that the distinction between past, present, and future is only an illusion, albeit a persistent one,” as he put it. (For this, Karl Popper called him “the Parmenides of modern physics.” Smolin, too,



Prague Orloj. The medieval astronomical clock on the wall of Old Town Hall, Prague.

quotes this letter, though he also claims evidence for a more Heraclitan Einstein, in some remarks reported by the philosopher Rudolf Carnap.)

Time Reborn
From the Crisis in
Physics to the Future
of the Universe

by **Lee Smolin**
Houghton Mifflin Harcourt,
Boston, 2013. 351 pp. \$28.
ISBN 9780547511726.

On the other side, some Heraclitans are so sure of their ground that they insist that if Einstein is right—if the distinction between past, present, and future is not objective—then time itself is an illusion. Accordingly, they interpret the block view as the claim that time is unreal. In a celebrated 1951 paper, philosopher D. C. Williams (following Wyndham Lewis) called these folk the “time snobs”: “They plume themselves that ... they alone are taking time seriously” (3).

To Parmenideans such as Williams and myself, this attitude is just linguistic imperialism, cheeky and rather uncharitable. Of course we believe that time is real, we insist. (It is as real as space is, the two being simply different aspects of the same four-dimensional manifold.) What we deny is just that time comes carved up into past, present, and future.

I’ve mentioned this because Smolin is a classic time snob, in Williams’s terms. When he says that he is defending the unpopular view that time is real, he means time in the

time snobs’ proprietary sense. This makes him sound like a defender of common sense—“of course time is real,” the reader is being invited to think—whereas in fact the boot is on the other foot. It is Smolin’s view that trips over common and scientific sense, denying the reality of what both take for granted.

To explain why, and to bring these issues down to Earth, suppose that I ask you to tell me what you did last week. You tell me a little about what happened to you, what you did, on each of those seven days. I can press you for more details, but can I complain that you haven’t told me which day (or minute, or second) last week was the present moment? Obviously not: each moment was present when it happened, but they all have this feature in common—no single moment is distinguished in any way from all the others. Have I denied that there was time last week? Again, obviously not. Like any other week, it contained 168 hours of the stuff! And the week wasn’t static—events happened, things changed.

So we can make perfectly good sense of time without picking out one moment as the present moment. We do it all the time, when we think about other times. And there’s nothing more radical to the block universe view than the idea that science should describe reality in the way that we just imagined describing last week, leaving out altogether the idea of a present moment, and hence a division between past and future. For the universe, as for last week, this is perfectly compatible with thinking that time is real, that events happen, and all the rest of it.

Again, Williams was beautifully clear about this, in his challenge to the time snobs. Here he is, pointing out that nothing that matters is missing from the block view:

Let us hug to us as closely as we like that there is real succession, that rivers flow and winds blow, that things burn and burst, that men strive and guess and die. All this is the concrete stuff of the manifold, the reality of serial happening, one event after another, in exactly the time spread which we have been at pains to diagram. What does the theory allege except what we find, and what do we find that is not accepted and asserted by the theory? (3)

Thus the block picture is simply a view of time from no particular time, just as a map depicts a spatial region from no particular place. (In both cases, we can add a red dot to mark our own position, but the map is not incomplete without one.) It no more makes time unreal than maps make space unreal.

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To deny this, to squeeze the obvious fact that we can talk about other times (like last week) into their peculiar present-centered cosmology, Heraclitans need to resort to desperate measures. They need to insist that when we seem to be talking about last week, or Einstein, or the history of the universe since the Big Bang, we are really talking about something in the present moment—our present evidence, perhaps.

Some brave Heraclitans are prepared to go this far, and Smolin may be inclined to follow them. His view is that “[t]he past was real but is no longer real. We can, however, interpret and analyze the past, because we find evidence of past processes in the present.”

But this is a little slippery. What exactly are we interpreting and analyzing, and what is this evidence supposed to be about? Like other Heraclitans, Smolin faces a dilemma. If history (the study of the past) can be taken at face value, as attempting to describe something other than the present moment, then it's a model for the block view of the universe in the way I have indicated—and shows how misleading is the claim that such a view is timeless. If not, then the proposal is much more radically revisionary, much more out of line with common sense, than Smolin seems to appreciate.

True, Smolin would not be alone in going to this extreme. At the Perimeter Institute

meeting where I first met Smolin, a distinguished quantum theorist insisted that the past is just a model we invent to make sense of present evidence and not to be taken literally (4). As I remarked at the time, this kind of attitude soon gets out of hand. I admitted a temptation to conclude that the distinguished speaker along with the rest of the audience were just aspects of a model I had constructed to make sense of my own evidence (no more to be taken literally than talk of what we all did yesterday). The time snobs' chauvinism of the present moment slides easily into solipsism.

So I have grave doubts whether Smolin's version of the Heraclitan view is any more plausible than its predecessors. And yet it has a tragic quality that other versions of the view mostly lack, for Smolin regards it as the gateway to promising new physics. I haven't said anything about these interesting ideas, which I'm not so well qualified to assess, but the tragedy lies in the fact that they seem entirely compatible with the block universe view, properly understood. The main idea is that the laws of physics can change from one time to another, and (leaving aside any special issues about the mutability of laws) we saw that the block universe can accommodate change as well as its rival. “Rivers flow, winds blow, ... men strive and guess and die,” as Williams puts it. Laws change too, if Smolin is right,

but this too would then be part of “the concrete stuff of the manifold, the reality of serial happening, one event after another” (3). So, Smolin's campaign against the Parmenideans is entirely unnecessary, a great waste of energy and brain hours.

I think Smolin would reply that it is essential to his proposal that the way in which laws evolve not be deterministic, that there be genuine novelty, and that the block view doesn't allow this. But although many people assume that the block picture is necessarily deterministic, this is simply a mistake (“albeit a persistent one,” as Einstein might have put it). There is no more reason why the block view should require that the contents of one time be predictable from knowledge of another than it is somehow a constraint on a map of the world that the topography at the Tropics be predictable from that at the Equator. I can think that there's a fact about what I had for lunch last Tuesday (or will have, next Tuesday) without holding that there is any decisive present evidence about these matters, such that Laplace's demon could figure it out based on what he could know now.

So in my view, Smolin is fighting an enemy with whom he has no real reason to disagree. He opposes a conception of time that if not actually on his side is certainly not in his way. And in waging this wholly unnecessary battle he aligns himself with a team whose own weaknesses (and misunderstandings of their rival) have been apparent for decades. If I'm right, then he's shooting himself in the foot, big time.

Coming back then to my opening question: Do my parts feel in peril in *Time Reborn*, and should I now be feeling sorry for the string theorists? Although I wish I could answer “yes,” my main sense isn't peril but sadness: sadness for the unnecessary of Smolin's campaign as well as sadness (and some embarrassment) that my home side—I mean team philosophy, now—has not succeeded in making these things clear. [“Must do better,” as a distinguished Oxford colleague put it (5).]

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BROWSINGS

Reducing Gun Violence in America: Informing Policy with Evidence and Analysis. Daniel W. Webster and Jon S. Vernick, Eds. Johns Hopkins University Press, Baltimore, MD. 2013. 310 pp. Paper, \$9.95, £5. ISBN 9781421411101.

One month after the December 2012 shooting in Newtown, Connecticut, killed 20 children and 6 adults, Johns Hopkins University convened a summit of gun violence researchers from around the world. Recognizing that the conversation about gun control in the United States had changed, the organizers aimed to have new policy be informed by the best available science. To that end, they produced this collection of 19 essays from 34 contributors in just 10 days. Most assess aspects of past efforts to reduce gun violence in U.S. states or test common prescriptions against existing research. Collectively, they highlight the problems of identifying individuals at high risk of violent crime and then realistically preventing them from accessing firearms. Four look abroad for lessons on how to reframe firearm regulation in response to gun violence. Australia, another country with a thriving gun culture, overhauled its laws in 1996 shortly after a lone gunman killed 35 people in Tasmania. The Dunblane massacre in Scotland (also 1996) led to the ban of all handguns in Great Britain within 2 years. Brazil was mobilized not by a horrific tragedy but by its staggeringly high homicide rate. In each country, new, evidence-based policies successfully reduced citizens' risk of death by gunshot. The volume ends with a predictable consensus of recommendations: limit sales to high-risk individuals and of high-risk firearms, improve background checks, and tighten regulation of gun sales.

In the 8 months since the Newtown shootings, federal attempts to implement such recommendations have stalled. But co-editor Vernick is buoyed by steps taken in states (including New York, Maryland, Colorado, Connecticut, and California) as well as the level of public support for stricter gun laws documented in the book. As he noted in our interview, previous major changes have required more than one legislative season to enact.

Online

sciencemag.org

Podcast interviews with co-editor Jon Vernick and contributor Rebecca Peters (http://scim.ag/ed_6149).

—Meghna Sachdev

Confronting the Sorry State of U.S. Health

Ronald Bayer,* Amy L. Fairchild, Kim Hopper, Constance A. Nathanson

In January 2013, the U.S. National Research Council (NRC) and Institute of Medicine (IOM) issued *U.S. Health in International Perspective: Shorter Lives, Poorer Health*, a stunning depiction of how, over the past four decades, the comparative health status of Americans has declined (1). The report applied a term, commonly used to describe the relative deprivation of social groups, to the nation as a whole: the “U.S. health disadvantage.” How can this be explained and what is to be done?

Researchers have known about the role of social inequality in this decline, yet the public and policy-makers studiously avoid this challenge to Americans’ self-concept. The health status of Americans is a social problem that demands social solutions. Will this analysis from two of the most prestigious science advisory bodies in the United States become a turning point or be consigned to the dustbin of history, with other studies critical of U.S. health and health care?

The report ranks the United States last among peer nations in health status and compares it unfavorably to 17 peer countries at almost every stage of the life course. The United States has higher rates of adverse birth outcomes, heart disease, injuries from motor vehicle accidents and violence, sexually acquired diseases, and chronic lung disease. Americans lose more years of life to alcohol and other drugs. The United States has the highest rate of infant mortality among high-income countries and the second highest incidence of AIDS and ischemic heart disease, and it has, for decades, experienced the highest rates of obesity in children and adults and diabetes from age 20 onward. Only those who survive to age 75 escape this pattern. The report emphasizes socioeconomic causes—the “drivers”—of these outcomes.

From its origins in the early 19th century, the study of public health has been informed by concerns about how sharp social class differences produce serious health conse-



quences. Rudolph Virchow observed in 1848 that preserving health and preventing disease required “full and unlimited democracy” (2). For some, like Friedrich Engels, the relation between health and wealth fueled a call for revolutionary change.

In contrast to such socialist proposals, the United States before the 1850s favored more conservative measures that would unfetter the market economy. Yet observers found themselves bewildered by a new contradiction: New York, the world’s richest city, also was burdened by its most miserable health statistics (3). The Citizen’s Association of New York launched a sweeping social and health survey (4). The findings provided grounds to create new, permanent public health institutions and sparked political action, which, by the early 20th century, culminated in the environmental, social, labor, and political reform movements that defined the Progressive Era in America (2).

By the eve of the First World War, however, broad social reform and regulation would be eclipsed by a narrower vision with twin targets: the germ and individual behavior. For bacteriologists, who commanded the field, education and therapeutic intervention were appealing as less costly and disruptive ways to change or prevent pathogenic behavior as compared with sweeping, ambitious social reforms that were seen as overreaching (5). During the Cold War, in which any allusion to left-wing thought was deemed “un-American,” questions about how social class and income might disadvantage whole groups risked being tarred as subversive and became too charged to ask (6). John Knowles, in 1977, captured the transformation: “Over 99 percent of us are born healthy and suffer premature

President Obama should convene a National Commission on the Health of Americans to address the social causes that have put the U.S.A. last among comparable nations.

death or disability only as a result of personal misbehavior and environmental conditions.” The average American can either “change his personal bad habits or stop complaining. ... Beneficent Government cannot—indeed, should not—do it for him ...” (7).

As the individual-focused approach was pursued in the United States, studies from England gave new life to the more-than-century-old understanding of the relation between wealth and health. In a nation with a much-praised universal health care system that assured access to all who needed services, the first Whitehall study demonstrated that rates of mortality were arrayed on a continuum—a social gradient of health—rather than being discontinuous or clustered. Those slightly better off socially were consistently at a health advantage compared with those of lower status. In 1980, England’s landmark Black Report examining the social gradient concluded: “Thirty years of the Welfare State and the National Health Service have achieved little in reducing social inequalities in health” (8, 9). This was not just the 19th-century picture of privilege versus poverty.

It is surprising that it was not until 2006 that the first epidemiological study comparing England and the United States found a social health gradient in both. What was unanticipated was that America as a whole was sicker than England (10). Even those at the top of the U.S. social ladder, despite their access to a vast and costly health-care system, fared worse than their British counterparts. An extended analysis (11) compared the United States to 11 European countries. “Americans face a health disadvantage” that “is remarkably pervasive and affects even the wealthy but is largest for the poor” (11).

To look deeper, the National Institute on Aging, part of the U.S. National Institutes of Health (NIH), requested that the NRC investigate international trends in life expectancy for those older than 50 years. The resultant 2011 study (12), noted that U.S. life expectancy at birth was 68.9 years in 1950, ranking 12th in the world. Despite an increase to 79.2 years, America’s relative standing had dropped to 28th by 2009. But the report and attendant press coverage focused on two explana-

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tory risk factors: smoking and obesity; this kept attention trained on individual health behavior.

The 2011 NRC report (12) set the stage for the NIH Office of Behavioral and Social Science Research, despite internal resistance, to commission a joint report from the NRC and IOM to see whether the comparative standing of the United States among younger Americans had also declined. It had. In explaining the patterns of morbidity and mortality that distinguish the United States from its peers, *Shorter Lives* (1) first addressed obvious disparities in health care provision. But it then pivoted to stress the limits of that focus: “Even if health care plays some role, decades of research have documented that health is determined by far more than health care” (1).

What then about health behaviors, which had figured so prominently in the 2011 NRC report on older Americans? Why were adverse behaviors more common in the United States than in other countries? Drawing upon a vast literature in social epidemiology, there was, argued *Shorter Lives*, a need to “look beyond individual behaviors and choices” to “systemic processes that may influence multiple health outcomes” (1).

The report explored the possible relation between the decline of the United States on various health measures and the co-occurrence of the worsening of some economic and social conditions since the 1970s. Although it could not assert a definitive causal relation, it argued that it was critical to examine the rise in income inequality (which some economists have described as being greater than at any point in the past 100 years), poverty, single-parent households, divorce, and incarcerations (1). It also explored causes from the role of individualism in American social ideology to the structure of the welfare state and patterns of gun ownership.

This bold shift to the social and structural and a sense of urgency sets the current report apart from its 2011 companion. While acknowledging the need for further research into sources of the U.S. disadvantage and into policy alternatives, its authors argued that action not be delayed by investigations that could well take years to complete. In so writing, the committee echoed a line from Goethe that appears on the title page of every IOM report: “Knowing is not enough; we must apply. Willing is not enough; we must do.”

It is not surprising that NIH all but ignored this report (13). This can, in part, be explained by NIH’s research vision, which Sandro Galea, former president, Society for Epidemiological Research, has described as

“focused heavily on resolving ever narrower molecular questions” (14). Nor is it startling that the institutionally cautious IOM and NRC exhibited little enthusiasm for sparking national discussion. A delayed public forum on the report in March 2013 did not include a panel on policy implications (15). It was the dogged effort of the committee leaders that paid off in widespread news coverage. “US Health is Lousy Compared with Peer Nations,” *The Los Angeles Times* reported (16). Other papers with international circulations were just as blunt; *The Atlantic* headlines described the United States as “dead last” (17).

Given the austerity-driven political stalemate that characterizes Washington today, combined with congressional attacks on federal support for social science research (18), it is hard to imagine a less propitious moment for confronting the profound challenge posed by *Shorter Lives*. In 1980, when the Black Report underscored the depth of class disparities in health, the newly elected Thatcher government all but buried it. “The term ‘inequalities’ could not be used,” wrote Sir Liam Donaldson, who would become England’s Chief Medical Officer (19). “Only subsequently was the term ‘disparities’ permitted” (19). Professional scrutiny and (later) commercial publication ensured that the Black Report became a critical turning point in health-policy discussion. In the UK, the Blair and Brown governments made serious commitments to incorporating the lessons of intervention research to address the social determinants of health, reduce inequalities, and improve overall health status (20).

Mobilization of an unprecedented kind is now necessary in the United States. It requires a campaign to remove the public veil of ignorance about the evidence. Notably, the NRC-IOM committee, although it had the authority to call for presidential leadership, felt constrained to assign this task to the philanthropic and advocacy community. Although they should have a role, given the stakes and the specter of official neglect, it is remarkable that government was not charged with the responsibility of spurring and sustaining a national conversation about what needs to be done.

We believe the gravity of the situation demands presidential action. In 1974, in the face of mounting evidence that abuse of human research subjects was commonplace, the National Commission for the Protection of Human Subjects and Biomedical and Behavioral Research issued the Belmont Report, which provided the principled basis for sweeping change in research norms. A decade later, the AIDS epidemic compelled President

Reagan to create the Presidential Commission on the Human Immunodeficiency Virus Epidemic. It successfully swam against powerful conservative currents and marked the beginning of action not only to fund research and care but also to prohibit discrimination against those with AIDS.

President Obama recently declared that growing social inequalities are tearing at the social fabric of the nation (21). He must now create a National Commission on the Health of Americans charged with holding public hearings and determining vigorous steps that must be taken to address, not simply the health of those at the bottom, but the comparative status of America as a whole. There is a strong evidentiary basis for action beyond interventions at the individual behavioral level (22–25). It is time to reverse a course of events at least four decades in the making.

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ASTRONOMY

The Curious Behavior of the Milky Way's Central Black Hole

Jeremy D. Schnittman

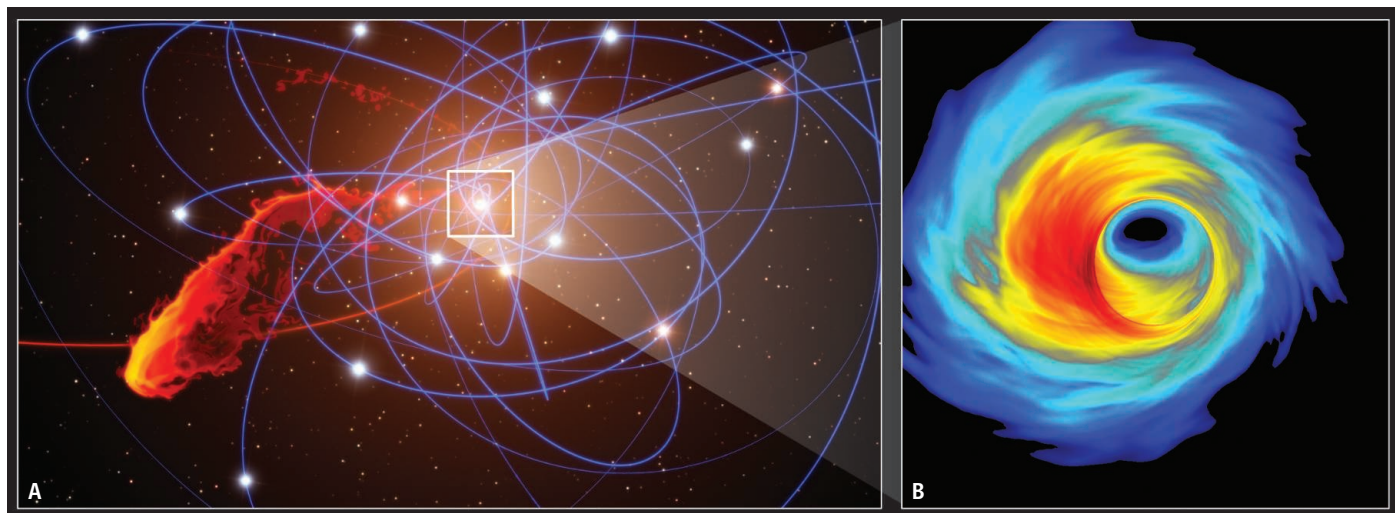
Over the past two decades, astronomers have assembled an incontrovertible body of evidence supporting the claim that a supermassive black hole resides at the center of our Milky Way galaxy (1). Thanks to its relative proximity, this black hole, named Sgr A* (Sagittarius A-star), provides an ideal laboratory for testing many of our theories about general relativity and extreme gravity. Because black holes are

40 million km (4). From observations of the rapid variability of x-ray flares from Sgr A*, we know that the x-ray-emitting region must be a comparable size (5) (see the figure, panel B). Combined, these vital stats can only be explained with a massive black hole.

These observations are tremendously challenging for a number of reasons. One is that the galactic center is swathed in a thick cloak of dust, which prevents any vis-

X-ray observations of the supermassive black hole at the galactic center help explain the unusually inefficient accretion of surrounding gas.

limit on angular resolution, a telescope must have a perfect form and be polished within a fraction of the wavelength of the light it is detecting. Although this level of precision is relatively easy to achieve for radio and even optical telescopes, it is currently impossible for the much shorter wavelength x-rays. The all-time champion of angular resolution among x-ray telescopes is the Chandra X-ray Observatory, which can resolve images with



Zoom into the galactic center. Orbiting within one light-year of the supermassive black hole Sgr A*, there reside thousands of stars and gas clouds (A). When

gas gets within a few black hole radii, it forms a hot torus of highly magnetized, relativistic plasma (B), emitting a broad spectrum of light from radio up to x-rays.

indeed black, we must infer their properties by observing the light emitted by hot gas immediately surrounding them. On page 981 of this issue, Wang *et al.* (2) present x-ray observations of Sgr A* that allow us to do exactly that, and help constrain some of the leading theoretical models for the behavior of material accreting onto the giant black hole.

By carefully tracking the orbits of individual stars around Sgr A* with the Keck telescope, we can infer its mass, with a best estimate of roughly 4 million times that of the Sun (3) (see the figure, panel A). With the use of a global network of radio telescopes, the physical size of Sgr A*'s source has been constrained to have a diameter less than

ible light from escaping, and thus makes it impossible to view with conventional optical telescopes. Infrared light does not suffer this diminution, so is the natural choice for large ground-based telescopes. However, because of its longer wavelength, it is harder to make sharp images with infrared light, so only the largest telescopes on Earth can resolve the individual stars orbiting around Sgr A*. Radio waves are even longer, yet radio telescopes can achieve remarkable resolution when combined in a network the size of the planet. X-rays, by contrast, have extremely short wavelengths, so should be ideal for taking high-resolution pictures.

The traditional challenges with x-ray astronomy are (at least) twofold: Earth's atmosphere absorbs x-rays, so any x-ray telescope must operate in space, inflating its cost and limiting its size; and, to reach the theoretical

better than 1 arc sec (1") detail. While impressive, this is still appreciably worse than the infrared and radio observations, which can resolve images better than a hundred times sharper.

As shown by Wang *et al.*, the x-ray emission from Sgr A* can be described as the superposition of a pointlike source from the black hole itself, and a much larger extended cloud of emission about 2" across. Within this cloud, we can identify over a hundred individually resolved bright stars, and infer thousands more that are too dim to detect (1). Many of these stars are constantly spewing out hot gas through stellar winds orders of magnitude stronger than that of our own Sun. By simply balancing the gas pressure from this wind with the gravitational pull of the black hole, we know that any gas within about 4" of Sgr A* should eventually get sucked in

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and accreted onto the black hole (6). Summing over the total wind loss from the surrounding stars, this gives an accretion rate of 10^{-5} solar masses per year (7).

The mysterious thing is, this accretion rate should make Sgr A* more than a million times brighter than it actually is. So either the vast majority of the gas never reaches the black hole, or it is somehow accreting so inefficiently that it never gives off any energy on its way to the horizon. It is as though you filled up your car's gas tank, drove 2 feet, and ran out of gas. Is there a massive leak in the fuel line, or is the mileage really that horrible?

With the unprecedented spatial and energy resolution of these new Chandra observations, Wang *et al.* can infer the temperature and density profile of the gas cloud surround-

ing Sgr A*. They show that over 99% of the gas never reaches the central black hole, but rather is ejected from the system, consistent with the predictions of a popular class of theoretical models called RIAFs (radiatively inefficient accretion flows) (8). By measuring the relative strength of various emission lines, they can also rule out a recent proposal that suggests that the diffuse x-ray emission is coming from the superposition of thousands of coronal flares from rapidly rotating stars around Sgr A* (9).

Another major challenge with observing Sgr A* has been its inherently low luminosity, orders of magnitude below its theoretical potential. And yet the future looks bright, quite literally. In the next few months, a large cloud of gas is on course to collide with the

black hole (10), which could potentially brighten by a factor of a million or more, greatly enhancing our ability to observe this unique region where gravity rules supreme.

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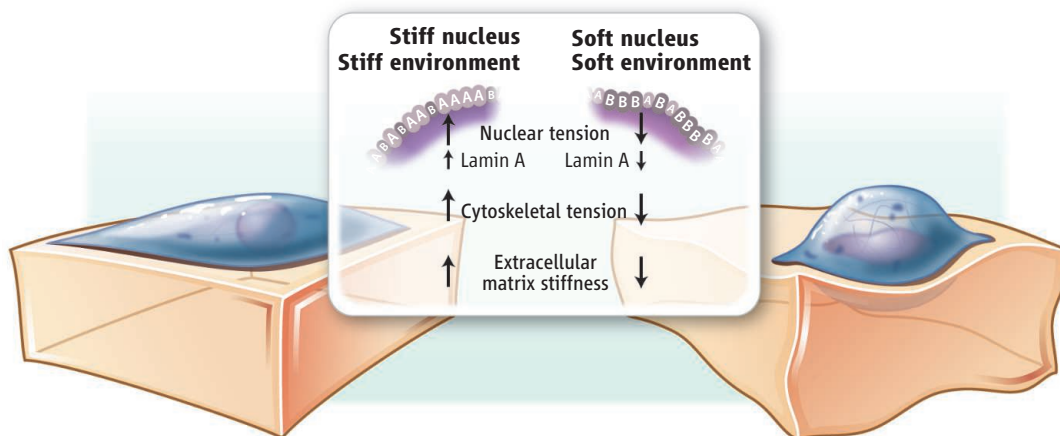
CELL BIOLOGY

Strength Under Tension

Russell Bainer^{1,2} and Valerie Weaver^{1,2,3,4,5,6}

Mechanics control gene expression to modulate tissue-specific differentiation, but the molecular mechanisms that underlie these effects remain unclear. On page 975 of this issue, Swift *et al.* (1) link tissue specificity and extracellular matrix stiffness to the relative abundance of the nuclear envelope protein lamin A. The findings support the idea that mechanical links exist between the nucleus and the extracellular microenvironment that direct cell fate, and imply that force mediates these effects by altering the biophysical properties of the nucleus.

Mechanical forces are generated at the cell and tissue level through cell-cell and cell-extracellular matrix interactions. Cells



Mechano-response. Tension from the extracellular matrix affects cytoskeletal tension on the nucleus. This affects the turnover of lamin A in the nuclear envelope, expression of *LMNA*, and stiffness of the nucleus.

sense, translate, and transmit mechanical cues from their periphery to the nucleus and induce changes in gene expression (2). This is accomplished in part by receptor-mediated tuning of biochemical and transcriptional circuits. Alternatively, mechanical cues can transmit forces through physical links between the nuclear membrane and the extracellular space. These connections distort the nuclear envelope and evoke transcriptional changes by locally altering the spatial accessibility of chromatin to transcriptional regulators (3, 4). Such changes occur rapidly and proportionately to the extent of the deformation (5), but histone deacetylase activity eventually increases after prolonged stress. This modification of histones condenses

chromatin, thereby modifying transcription. Swift *et al.* show that the force environment can change transcription of the gene *LMNA* and stability of the encoded protein, lamin A/C, to alter nuclear rheology. This indicates that mechanically driven cell differentiation involves interdependent changes in nuclear composition and transcriptional state.

Cells not only regulate their cytoskeletal organization, cell shape, polarity, and molecular state but also modify the extracellular matrix composition and topology to achieve tensional homeostasis. In this manner, cells can both sense and dictate the physical properties of their microenvironment while preserving the structural continuity within the surrounding tissue. Consequently, tissues

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acquire stiffness optima as an emergent property of the physical and biochemical interactions between their constituent cells. This tensional equilibrium confers macroscopic compliance properties that are critical for processes such as stem cell differentiation, embryonic development, and tissue homeostasis.

The specific mechanisms by which individual cells physically remodel themselves to functionally drive macroscopic changes in tissue compliance are not well understood, however. In addition to responding dynamically to immediate physical cues, cells must integrate mechanical signals to alter their long-term molecular state and cellular phenotype. Accordingly, such mechanisms must operate on long time scales to promote phenotypic stabilization, likely by directing transcriptional and epigenetic changes.

Swift *et al.* show that the relative abundance of lamin A is a key component of mechanoreciprocal responses and a major determinant of cell and tissue stiffness (see the figure). The authors observed that increased cell tension reduces the turnover of lamin A in the nuclear lamina. This causes accumulation of the mechanosensitive Yes-associated protein (YAP), a master transcriptional regulator. An increase in lamin A also triggers the serum response factor (SRF) signaling pathway, whose gene targets control the actin cytoskeleton. The accumulation of lamin A also drives translocation of the retinoic acid receptor into the nucleus to stimulate transcription of *LMNA* and the production of more lamin A. The findings suggest a mechanism that could explain the strong correlation between relative abundance of lamin A in diverse cell types with macroscopic tissue stiffness. Interestingly, as the relative abundance of lamin A increases, the viscosity of the nucleus also increases. It is possible that in addition to activating mechanosignaling pathways, an increase in lamin A may play a role in physically stiffening the nucleus as part of the cellular response to increased tension.

The model presented by Swift *et al.* proposes how cells that are otherwise acutely sensitive to mechanical signals can structurally acclimate to tissue environments that are pervasively subject to a sizable mechanical load. In such circumstances, lamin A could physically reinforce the nuclear envelope, which would stabilize interactions between chromatin and the nuclear lamina and inure the cell to subsequent nuclear distortions that might otherwise occur in a high-tension tissue environment. Indeed, such an observation could explain why many tumors, which are typically stiffer than the surrounding tissue

and are characterized by increased interstitial pressure, also often have greater amounts of lamin A compared to normal cells (6, 7). Moreover, the model hints at a deeper interplay between mechanosensitive signaling pathways, which are apparently affected by both external stress and elevated lamin A abundance, and transcriptional changes that are induced by direct tension on the nucleus from cytoskeletal contacts, which are likely to be modified in cells with lamin A-rich nuclear envelopes. Alternatively, increased tissue-level stiffness might compensate for lamin A-induced changes in nuclear compliance, such that the cytoskeleton simply transmits a greater mechanical load to enable cytoskeletal contact points on the nucleus to remain mechanically sensitive.

The provocative feed-forward mechanism governing lamin A concentration in the model of Swift *et al.* is critically dependent on retinoic acid receptor activity and provides another potential layer of mechanosensitive regulation. For instance, in the absence of ligand, the retinoic acid receptor will heterodimerize with one of the nuclear receptor co-repressor repressor proteins which, together with their associated histone deacetylases, inhibits transcription from spe-

cific promoters to maintain heterochromatin. Given the importance of the nuclear lamina in stabilizing heterochromatin, the data presented by Swift *et al.* implicate epigenetic silencing of lamina-associated chromatin as a key component of tension-mediated transcriptional regulation.

Additional studies are required to explicitly link mechano-reciprocal nuclear stiffening to gene-regulatory mechanisms. Elucidating the phenomena that collectively define cellular mechanosensation will also require studies to more completely unify the functionality of direct and indirect force-mediated transcriptional mechanisms. For cells, it seems that tension can become a source of considerable strength.

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GEOPHYSICS

Minimizing Caribbean Tsunami Risk

Christa von Hillebrandt-Andrade

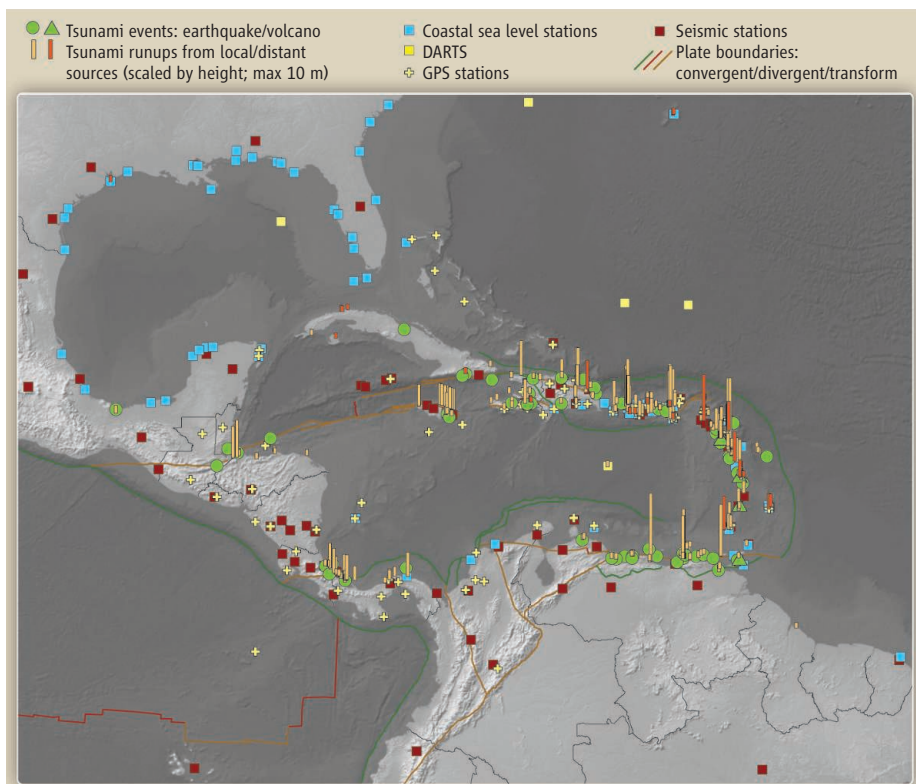
Efforts to understand and respond to tsunami risks in the Caribbean are beginning to bear fruit.

In the past 500 years, more than 75 tsunamis have been documented in the Caribbean and adjacent regions. Since 1842, 3446 people are reported to have perished to these killer waves. The tsunami generated by the 2010 Haiti earthquake claimed several lives, but the most recent devastating events were the 1946 tsunamis of the Dominican Republic, with at least 1800 victims (1). Since then, there has been an explosive increase in residents, visitors, infrastructure, and economic activity along Caribbean coastlines, increasing the potential for human and economic loss. On any day, more than

500,000 people could be in harm's way along the beaches (2), with hundreds of thousands more working and living in the tsunami hazard zones.

In the Caribbean, most tsunami events are very short-fused: The waves can reach the shores within minutes of an earthquake, volcanic eruption, or submarine landslide. Addressing this threat requires a very effective monitoring and warning system, as well as a public that is acutely aware of the natural signs of an impending tsunami. The current performance goal is that earthquakes with moment magnitudes $M_w \geq 4.5$ in the Caribbean should be detected within a minute and that the tsunami warning centers issue a message within 5 min, which is immediately relayed by the local authorities to the public in case of threat (3). Tsunami evacuation maps

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Early warning required. Tsunamis up to 10 m in height have been observed throughout the Caribbean; many reach shores within minutes. An extensive network of National Ocean Service, GLOSS (Global Sea Level Observing System) and other coastal sea level stations, DART (Deep Ocean Assessment and Reporting of Tsunamis) buoys, COCONet GPS stations, and seismic stations contributes to the local tsunami and coastal hazards warning system CARIBE EWS. [Data from NOAA/National Geophysical Data Center]

indicate where people should move to in the case of a tsunami event. Land-use planning and protection of coastal resources are also important for mitigating tsunami impacts.

Since the mid-1990s, the Intergovernmental Oceanographic Commission of UNESCO has advocated the development of a Caribbean tsunami warning system. In the wake of the 2004 Indian Ocean tsunami, it succeeded in establishing an intergovernmental coordination group tasked with developing a tsunami and other coastal hazards warning system for the Caribbean and adjacent regions (CARIBE EWS) (4). The CARIBE EWS includes 32 member states and 16 territories and commonwealths (see the figure).

In 2006, when the CARIBE EWS met for the first time, there were just a dozen seismic stations, a handful of sea level stations, some tsunami inundation maps, a few educational resources, one country with a tsunami warning protocol, and no evacuation maps in the region.

Similar to other tsunami-sensitive regions in the world, the tsunami detection capabilities have increased dramatically since then. Although seismic data are used to detect earthquakes that could generate tsunamis,

sea level data are currently used to both confirm the size of the tsunami and forecast its impact. Today, there are more than 115 seismic stations and 55 sea level stations in the Caribbean and adjacent regions (see the figure). Global Positioning System (GPS) data could also help to provide accurate and timely tsunami warnings (5). The CARIBE EWS has endorsed projects like the Continuously Operating Caribbean GPS Observational Network (COCONet), which has already installed, refurbished, or made available data from more than 84 GPS stations in the region (6). The augmentation of observational data has helped reduce the detection time of earthquakes by 3 to 4 min to 1 min (7).

Unlike the UNESCO regional tsunami warning systems for the Pacific and Indian Oceans and the Northeastern Atlantic and Mediterranean, no Tsunami Warning Center has yet been established in the Caribbean. Nevertheless, forecasts and warnings are being provided on an interim basis by the

U.S. National Weather Service (NWS) Tsunami Warning Centers in Alaska and Hawaii. In 2010, the NWS established the Caribbean Tsunami Warning Program (CTWP) in Mayagüez, Puerto Rico, as part of its three-phased approach to the establishment of a tsunami warning center in the region. Given significant advances in phases one (education) and two (monitoring), NOAA is currently evaluating whether to proceed with phase three and establish a Regional Tsunami Warning Center at the University of Puerto Rico Mayagüez or to consider proposing an alternative end state that also ensures an equal or improved level of service.

Tsunami inundation and evacuation maps help guide people to safety during a tsunami emergency. These maps require as input detailed information on tsunami sources, as well as high-resolution digital elevation models (DEMs). Only a few of the countries in the region have had the resources to undertake this work. Efforts are underway to develop a database on tsunami sources and improve the DEMs that could also be used for other hazard and risk studies.

Currently 94% of all countries and territories of the Caribbean have a designated tsunami contact and warning points for coordinating the system and issuance of alerts. Tsunami exercises are conducted to test communication between warning centers, warning points, and the public, as well as practice and review emergency response procedures. Regional exercises were conducted in 2011 and 2013. For the 2013 exercise,

almost 50,000 people and 500 organizations participated from 45 of the countries and territories in the region. Given the success of these exercises in helping to identify and correct

deficiencies and sharing of best practices, it has been decided that such exercises should be conducted annually (8).

Community-based recognition programs help to motivate, harmonize, and track readiness. One such initiative is the TsunamiReady Program of the NWS, also supported internationally as a pilot project with the Intergovernmental Oceanographic Commission (IOC). The program requires the designated communities to have 24/7 warning capabilities, evacuation maps and signs, exercise and outreach activities, and emergency response plans. In addition to more than 30 coastal communities in Puerto Rico, Anguilla was the first non-U.S. jurisdiction

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to complete the requirements. The Caribbean Tsunami Information Center (CTIC) in Barbados, established in 2013, will further support these and other efforts to increase tsunami resilience.

Although it now takes about a minute to detect sizable earthquakes and most countries have warning points and operational procedures in place, the time it takes to detect a tsunami and communicate its impact can be reduced further. Also, the tsunami warning system is primarily triggered by earthquakes detected by seismic stations; in the event of a volcanic eruption or landslide not associated with an earthquake, it is likely that no tsunami message would be issued based on current technology. This shortcoming is being addressed but will require advances in both

sensing and our understanding of non-seismically induced tsunamis. Furthermore, most coastal communities still require evacuation maps and signs, while all require the ongoing education of their residents and visitors. Significant headway has been made, but additional economic resources are still required to sustain and strengthen our warning system. By making these investments, we will not only save lives and protect livelihoods from tsunamis, but will be better prepared for earthquakes and other coastal hazards.

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Acknowledgments: The author is chair of the UNESCO IOC Intergovernmental Coordination Group for Tsunamis and other Coastal Hazards for the Caribbean and Adjacent Regions and the past president of the Seismological Society of America.

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MATERIALS SCIENCE

A Clear Advance in Soft Actuators

John A. Rogers

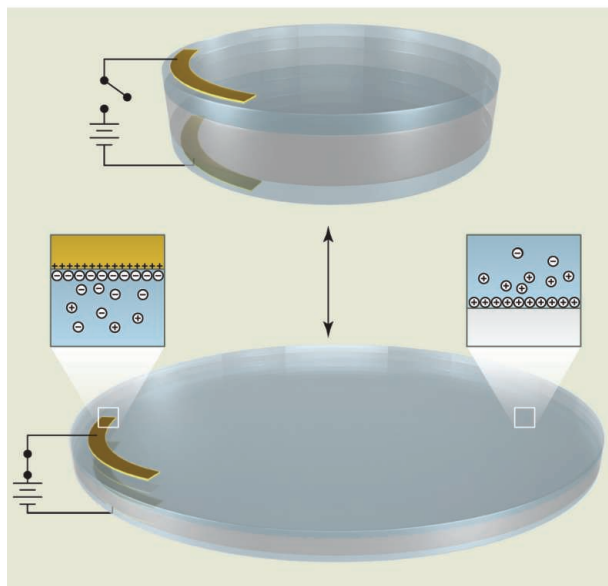
Development of actuator technologies with capabilities that can match or exceed those found in biology represents a topic of long-standing interest within the advanced robotics community. One promising and remarkably simple class of such an “artificial muscle” exploits a dielectric elastomer (an electrical insulator) sandwiched between a pair of mechanically compliant electrodes (1, 2). Electrostatic force generated by an applied voltage deforms the dielectric and causes rapid, controlled displacements with large amplitudes. On page 984 of this issue, Keplinger *et al.* (3) describe an important advance in this dielectric elastomer actuator (DEA) technology, in which the authors replace the electrodes with soft, ionic hydrogels. The result provides a clever solution to a daunting materials challenge; it enables delivery of high voltages for fast, effective operation without any mechanical constraint on the motions of the dielectric, in a form that also provides almost perfect optical transparency.

Films of carbon powder or grease loaded with carbon black served as electrodes for the earliest DEAs (1, 2). Although valuable for initial prototypes, such materials have poor reliability and are not readily compatible with established manufacturing tech-

niques. Improved characteristics can be achieved with alternatives based on sheets of graphene (4), coatings of carbon nanotubes (5), surface-implanted layers of metallic nanoclusters (5), and corrugated or patterned films of metals (5). These options yield working DEAs, but with limited mechanical properties, sheet resistances, switching times, and capacity to integrate into advanced actuator designs. The authors show that a different class of material (soft, transparent hydrogels) and a different mode of charge transport (ionic, rather than elec-

tronic) can yield electrodes with characteristics that are remarkably well suited for use in DEAs. A key but nonobvious realization is that even aqueous ionic hydrogels can deliver potentials of several kilovolts, despite the onset of water electrolysis at less than 1.5 V.

The physics is relatively simple. A potential applied to a conductor in contact with a hydrogel induces ionic transport that yields a net charge at the interface together with an adjacent screening charge, known collectively as an electric double layer. A cor-



Thin, stretchy transparent actuators. The hydrogel electrodes developed by Keplinger *et al.* carry current through ionic flow for use in soft, electrostatic actuators. In this artificial-muscle technology, sheets of hydrogels deliver large voltages to a dielectric elastomer. The applied potential creates electrical double layers that induce electrostatic forces to compress the elastomer. These deformations are well controlled, reversible, and capable of high-frequency operation. The resulting devices can be perfectly transparent, with potential for use in applications such as noise-canceling windows and display-mounted tactile interfaces.

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responding charge appears at the interface with the dielectric elastomer (see the figure). The enormous difference between the capacitance of the double layer and the dielectric leads to a potential across the dielectric that can be millions of times greater than that across the double layer. As a result, potentials in the kilovolt range can be realized in the DEA without electrochemically degrading the hydrogel.

High-frequency actuation is also possible. Careful analysis shows that switching speeds in practical systems are limited only by mechanical inertia. Furthermore, because the stiffness of the hydrogel can be thousands of times smaller than that of the dielectric, actuation can occur freely, without mechanical constraint. These attractive characteristics are complemented by an additional, interesting feature: Hydrogels can have exceptionally high optical clarity across the visible range, thereby opening up a range of application possibilities enabled by transparent actuators.

The authors formed DEAs with this design simply by laminating films of polyacrylamide hydrogels formed with salt water onto the surfaces of dielectric elastomers. Such actuators can change their dimensions by nearly a factor of 2 and switch with millisecond speeds. As a demonstration, the authors built loudspeakers that produce high-fidelity sound throughout the audible range. The thin, planar geometries of these devices, taken together with their nearly complete optical transparency, foreshadow interesting applications such as active noise-canceling windows and display-mounted tactile interfaces. Adaptive optics

represents another potential field of use. These and other prospects motivate the development of further refinements in the materials, including schemes to prevent drying of the hydrogels and methods to eliminate ionic build-up, hysteresis, and electrical shorting.

The success of ionic hydrogel conductors in DEAs hints at possibilities for their use in other unusual electrical systems, such as new classes of circuits and sensors that have elastic properties and shapes precisely matched to biological tissues for implants, surgical tools, and diagnostic systems that intimately integrate with the curved, dynamic external or internal surfaces of the body (6–10). Ionic hydrogels can offer favorable mechanics, and they can be biocompatible. Also, their operation exploits transport of ions, much like the intrinsic mode of electrical function in biological systems. Ionics, therefore, provides a natural type of biotic-abiotic interface.

Although the relatively slow speeds and the physical mass transport associated with ionic conduction preclude the general use of hydrogels as alternatives to metals in electronics, many possibilities can be considered. In fact, seminal experiments in the earliest days of semiconductor device research relied critically on ionic conductors to investigate field modulation of contact potentials in silicon and to enable the first solid-state amplifiers, as summarized in Bardeen's Nobel lecture in 1956 (11). Work in just the past 10 years has established the utility of similar electrolyte gate electrodes in printed and organic electronics (12). More recently, demonstration experiments showed that deform-

able ionic gels can serve as elements of high-performance, stretchable graphene transistors (13).

In the context of biomedical devices, related types of gels are already in widespread use for low-impedance interfaces between metal electrodes and the surface of the skin. One vision for system design might strategically combine both electronic and ionic modes of operation, in which the latter enables conformal electrical interfaces to biological tissues and provides soft mechanical actuation and sensing, whereas the former affords signal processing, control, acquisition, data storage, and transmission. Developing an associated base of fundamental knowledge in materials and device designs represents a promising direction for future work.

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PSYCHOLOGY

The Poor's Poor Mental Power

Kathleen D. Vohs

Few people wish to be poor. Many find it puzzling that those in poverty seem to get stuck in that state, even when there are opportunities to improve one's lot. On page 976 of this issue, Mani *et al.* (1) provide a possible reason: Poverty-related concerns impair cognitive capacity. Simply put, being poor taps out one's mental reserves. This could explain data showing that the poor are likelier than others to behave in ways that are harmful to health and impede

long-term success—in short, behaviors that can perpetuate a disadvantaged state.

The eye-opening study of Mani *et al.* included laboratory experiments and field studies that tested the “cognitive constraint” hypothesis. One experiment gave individuals who were poor (defined by household income) hypothetical financial decisions, followed by tasks that measured mental abilities. Poor people who earlier had contemplated a difficult financial decision showed worse mental performance than others. A study of farmers demonstrated that the mental acuity of the same person varied with swings in income. Farmers were given challenging cog-

Poverty's mental toll might explain its connection to unhealthy impulsive behaviors.

nitive tests before and after harvest. Before harvest, the farmers experienced much financial strain, whereas after harvest (and the receipt of payments), they did not. The results showed clear and demonstrable improvement in cognitive capacity after harvest. This outcome held after accounting for the stress of pre-harvest periods. The authors propose that poverty imposes a cognitive load, which impairs cognitive capacity.

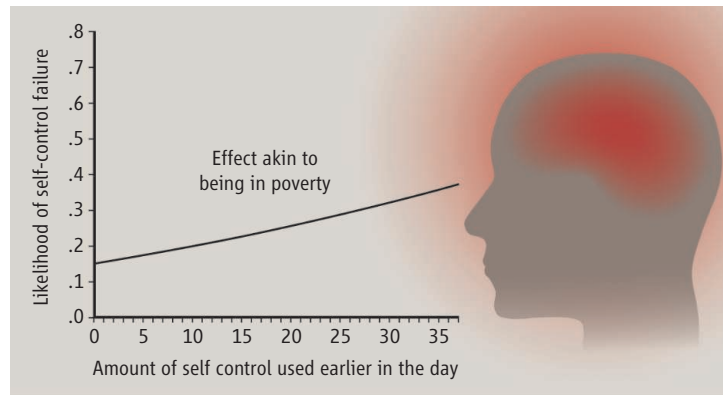
The depletion of mental functioning with poverty comports with a framework called the limited-resource model of self-control. Failures of self-control are implicated in some of society's most pressing problems,

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including poverty (1–6). When people want to reach a goal, they use self-control to produce responses and behaviors aimed at moving themselves from the current (undesirable) standpoint to the preferred state. This powerful process, however, is not used as often as it should be. One reason is that self-control is a limited and depletable resource (7). When people use self-control, it is like top-flight running for a cheetah, in which a brief period of exertion results in exhaustion.

Everyone must regulate eating and spending, and wearing down self-control resources leads to detrimental behaviors for both. In one study, people made to resist the lure of delicious chocolates later showed worse performance on demanding mental tasks and at managing negative emotions. Moreover, it led to overeating unhealthy foods (8). In another situation, participants were given cash to spend or keep. Those who earlier had used self-control to suppress unwanted thoughts later spent more money and reported stronger desires to spend all the newfound cash. The depletion of self-control ability led to unwise spending (9). Both examples suggest a vicious cycle: Overcoming urges and making decisions can deplete mental resources, which in turn can lead to problematic behaviors. Because the poor must overcome more urges and make difficult decisions more often than others, they are more likely to overeat, overspend, and enact other problematic behaviors.

Self-control may be the greatest human strength (6) because it is involved in the ability to make wise choices. Several studies have found that after using self-control (and thus reducing the resource), decision-making patterns shift toward favoring intuitive over reasoned options (10). For example, options were constructed so that they were extreme on some dimensions (e.g., expensive and high quality) or balanced (moderate price and modest quality). Choosing the latter reflects the use of deliberate cognitive strategies to accept trade-offs. Those who earlier had engaged in self-control activities preferred extreme options that required fewer trade-offs. Moreover, the process of making trade-offs itself requires self-control (11). These findings suggest that decisions requiring many trade-offs, which are common in poverty, render subsequent decisions prone to favoring impulsive, intuitive, and



Mental toll. People become progressively worse at self-control the more they have engaged in self-control previously. The more that people used self-control not to give into desires earlier in the day, the more likely it was that a desire impelled an impulsive behavior later in the day. This situation is akin to poverty, which requires that people often battle back desires. Adapted from (12).

often regrettable options.

Regulating urges and desires, even basic ones such as for sleep and leisure, exacts a cumulative effect. Researchers surveyed people seven times a day for several days, tracking their recent desires, attempts at resistance, and whether they performed behaviors implied by the desires. In line with the limited-resource model, people became progressively worse at self-control the more they resisted unwanted desires (12) (see the figure).

Chronic pain may be analogous to poverty (13) as these patients' behaviors parallel those seen by Mani *et al.* Patients with fibromyalgia, a chronic pain disorder, performed a task that either did or did not require focused attention (comparable to the focus required to drive during pummeling rain, for example). Afterward, they were given a challenging cognitive task. The outcomes were striking. Patients with chronic pain had poor cognitive performance regardless of whether they earlier had used self-control or not. By contrast, healthy individuals showed the standard depletion effect of worse performance only after previous exertion of self-control. These findings imply that there may be entire segments of people who, like the poor and those chronically in pain, suffer constant self-control depletion.

The limited-resource model of self-control points to the following state of affairs for people in poverty. Resisting urges and controlling one's behavior drains self-control resources. The poor must resist and control more than others because they have less money, food, and expendable time. Such limited supplies demand trade-offs, and hence many decisions. And, there is a snowballing, adverse effect of engaging in self-control on subsequent self-control capacity. Altogether, these processes spell a dwindling

supply of self-control with few chances to recover.

Governments and organizations must recognize that the lives of the poor are filled with land mines of desire, trade-offs, and self-control dilemmas. Paring down the sheer volume of decisions that the poor must make—perhaps through defaults—and allowing others to share in the decision-making process could help. Scheduling interviews and appointments earlier in the day could be beneficial because people generally possess greater cognitive capacity at that time (12). Public

settings that require individuals to handle forms, rules, and decisions could have a care area for children to minimize competing demands on attention.

Recent estimates show that about 20% of the world's population is in poverty (14). Although that is half of what it was 20 years ago, it is nonetheless a huge number (14). Economists are fond of the theory that the more people on Earth, the better, because people create ideas. With more people come greater odds of discovering the cure for cancer, renewable energy sources, or how to cultivate world peace. That premise rests on the notion that all people have adequate mental capacity, a premise now called into question by Mani *et al.* for a fifth of the world's population.

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IBI* SERIES WINNER

An Inquiry into the Water Around Us

Erin. K. H. Saitta,^{1,2*} Tamra Legron-Rodriguez,² Melody A. Bowdon^{2,3}

An Inquiry into the Water Around Us, an IBI prize-winning module, brings the college classroom into the community.

On a bright October morning, three young women in laboratory coats work together amid chemicals, instrumentation, and laboratory notebooks. The scene looks like a typical university chemistry lab, but two of the scientists are actually local high-school students analyzing water samples they have collected from around their community. Their undergraduate lab partner is completing the general chemistry lab module “An Inquiry into the Water Around Us,” which harnesses the intellectual power of three important pedagogical components: inquiry-based learning, service-learning, and instruction in scientific communication. The project brings science, technology, engineering, and mathematics (STEM) majors into direct collaboration with younger students to explore the critical community issue of water quality. This combination approach has the potential to promote recruitment of STEM majors, motivate underrepresented secondary (middle- and high-school) students to attend the university and pursue STEM careers, support retention of female STEM majors, and shape a more civically engaged population of future scientists through the inclusion of service-learning, a pedagogy that takes the college classroom into the community (1, 2).

The module’s centerpiece is a 2-month collaborative investigation between college students and their high-school lab partners, which brings together students on their respective campuses through interactive Web conferences that introduce and summarize the project. Once the project is launched, the secondary students collect water samples from local lakes, wells, and other community sources to investigate alongside the



Three students working on the laboratory experiment.

university students. Related experiments are performed separately at each institution, and one collaborative experiment is performed when the secondary students visit the university campus (see the photo). Each is intended to increase students’ understanding of chemistry concepts while encouraging scientific problem-solving, communication, and civic awareness.

Virtual communication tools create a unique learning opportunity and simplify service-learning logistics, such as scheduling and transportation (3, 4). Dialogue between the two populations takes place synchronously through Skype and/or FaceTime and asynchronously through personal letters. These details allow maximum student interaction and minimal travel, which gives the project a small carbon footprint and a large academic impact.

True to the nature of inquiry, the module focuses on the process of science and models authentic research practices in the classroom (5). The amount of guidance provided varies for each experiment, as activities are at many points on the inquiry spectrum. Experiments begin with key questions, and students are tasked with designing their procedures to collect evidence and make claims in response. The most-guided activity is the Analysis of Hard Water Ions, where students are provided with an “established method,” intended to mimic U.S. Environmental Protection Agency (EPA) methods used by envi-

ronmental chemists. The least-guided activities are Phosphate Analysis and Total Ion Concentration. In every experiment, students organize their results, construct meaning from their data, and make claims based on their evidence. Each set of lab partners investigates a unique local water sample that contributes to the overall analysis; aggregation of class data allows students to analyze data in context and observe trends that might have broad community implications.

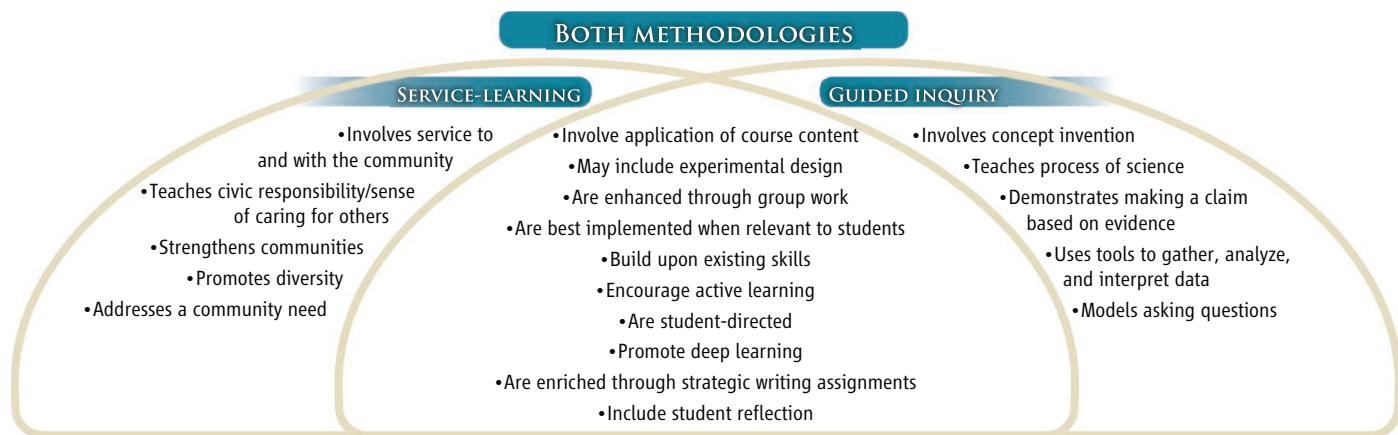
Service-learning engages students as co-investigators, giving both populations the opportunity to take ownership of the work. The module encourages undergraduate students to identify themselves as part of the scientific community, as well as their local community, and inspires them to use their knowledge and position in civically minded ways.

The module’s emphasis on informal and civically focused communication instruction encourages students to think about how scientists interact with their communities. Reading assignments include local newspaper articles and periodicals that discuss excess nutrients in local water systems and the debate over EPA regulations, framing the project within a broad context. Students are given writing assignments to explain scientific findings to their secondary school partners through personal letters. Instruction in this regard is critical: During the first iteration of the project, many of the university students’ letters summarizing the research results could not be sent because they included incorrect assumptions, unnecessary sensationalism, or inappropriate tone. After modifying the assignments, a strategic approach to writing instruction and practice was developed in the “Scientists Write!” portion of the curriculum. This weekly homework activity reinforces lessons about scientific communication and frames targeted writing assignments. Focusing on topics such as audience, purpose, and word choice, writing assignments are now

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The unique and overlapping fundamental themes (5–10).

integrated to complement and deepen the learning process.

Although inquiry and service-learning are extensively researched and widely implemented individually, there is currently no published dialogue on how the two can be applied simultaneously in a science lab. We have found that the two methodologies, particularly when supported by an emphasis on scientific communication, complement each other with overlapping themes as seen in the chart above. Both encourage active learning and the application of course material. Inquiry-based learning adds a focus on concept invention and the process of science, whereas service-learning contributes to students' civic awareness and underscores social relevance.

Standardized departmental content-mastery assessments indicate that the university students who have participated in the service-learning inquiry module learn chemistry content to at least the same extent as those in the inquiry-only curriculum. End-of-semester surveys indicated that 93% of students felt that the project helped them evaluate information, communicate ideas, and apply course

content in the real world. In addition, all 82 students who have completed the module thus far reported that the experience allowed them to engage in positive interactions with people from different social, economic, and ethnic backgrounds from their own.

Two key lessons learned from successful implementation of the module include

1) Well-managed ongoing and direct correspondence between the secondary and college students is crucial. Although our first iteration of the project included a single group-to-group virtual connection, learning outcomes and satisfaction levels for both populations were improved when students made multiple personal connections throughout the project.

2) Simply making a positive connection with secondary peers is not sufficient to help college students learn to communicate effectively. To maximize the student learning outcomes in content and communication, writing about science for a variety of audiences must be explicitly addressed in the course.

An Inquiry into the Water Around Us was designed to be a valuable addition to any general-chemistry inquiry curriculum. The experiments teach chemistry content

in a course that meets once a week and uses equipment that can be found in most laboratories. The inquiry activities investigate actual environmental samples and include social and political dialogue important to the local community. The service-learning component requires no outside travel for undergraduates yet serves as motivation for engagement.

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Supplementary Materials

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TRANSLATIONAL RESEARCH

Prize-Winning Genetics Research Yields Test for Prostate Cancer

One out of every six men in the United States will be diagnosed with prostate cancer in his lifetime. Many of these cases are detected with the so-called PSA blood test, but this test is prone to false positives and may be followed up with a more invasive biopsy.

Another option should soon be available in the form of a urine test, which could complement the PSA as a way to identify men at highest risk for prostate cancer. The test is based on the discovery by Scott Tomlins of the University of Michigan Medical School that two particular genes are fused together in almost half of all prostate cancers and are thus a useful marker for the disease.

The test is expected to be available within the year through the University of Michigan's CLIA-certified Michigan Center for Translational Pathology, according to Tomlins. Although the test is not yet approved by the Food and Drug Administration, the federal Clinical Laboratory Improvement Amendment, or CLIA, allows individual laboratories to offer diagnostic tests to patients, without FDA approval.

The discovery of this gene fusion is notable not only for its scientific and clinical

implications but also because the researcher behind it is just 34 years old. AAAS and the journal *Science Translational Medicine* on 15 July honored Tomlins' work with the inaugural AAAS Martin and Rose Wachtel Cancer Research Award. This \$25,000 prize recognizes outstanding work by young scientists performing breakthrough cancer research.

Tomlins' discovery "altered the way the field thought about the genetic causes of the common solid tumors—not only prostate but also lung, breast, and colon cancer," said Katrina Kelner, a member of the award selection committee and Editor of *Science Translational Medicine*.

When Tomlins began doing research at the University of Michigan, where he earned his M.D. and Ph.D. degrees, he was more interested in using DNA microarrays as a tool to study cancer generally. Lured by a free lunch, Tomlins attended a seminar by Arul Chinnaiyan, a University of Michigan scientist who had just published one of the first studies using microarrays to study prostate cancer. Chinnaiyan eventually became Tomlins' scientific mentor.

Early-career coup. Identifying a marker for prostate cancer earned Scott Tomlins (left, with AAAS CEO Alan I. Leshner) a major AAAS award.

"Although I sort of landed in prostate cancer by chance, it's an enormous public health problem, and there are so many nuances to both the clinical management and molecular alterations that it's still incredibly interesting to work on," Tomlins said.

Prostate cancer is a leading cause of cancer death in American men, second only to lung cancer. Although the disease can be fatal, success stories are common, and many patients are cured by surgery or radiation.

Tomlins and his colleagues found that two genes named *TMPRSS2* and *ERG* are incorrectly glued together in almost half of all prostate cancers. In normal prostate cells, *ERG* is turned off and *TMPRSS2* is turned on. When parts of *TMPRSS2* in the prostate become fused to *ERG*, *ERG* is turned on. Once activated, *ERG* drives cancer development by inappropriately turning on or off other genes. This gene fusion is the most specific marker of prostate cancer yet to be found.

Tomlins' work on gene fusions spurred the development of the new test for prostate cancer, first published in the 3 August 2011 issue of *Science Translational Medicine*, which can detect the product of the gene fusion in patients' urine.

More recently, he and his colleagues have been working on another test, which combines the urine-based detection of gene fusions with the detection of elevated serum PSA levels.

—Nadia Ramlagan

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The Chemistry and Applications of Metal-Organic Frameworks

Hiroyasu Furukawa, Kyle E. Cordova, Michael O’Keeffe, Omar M. Yaghi*

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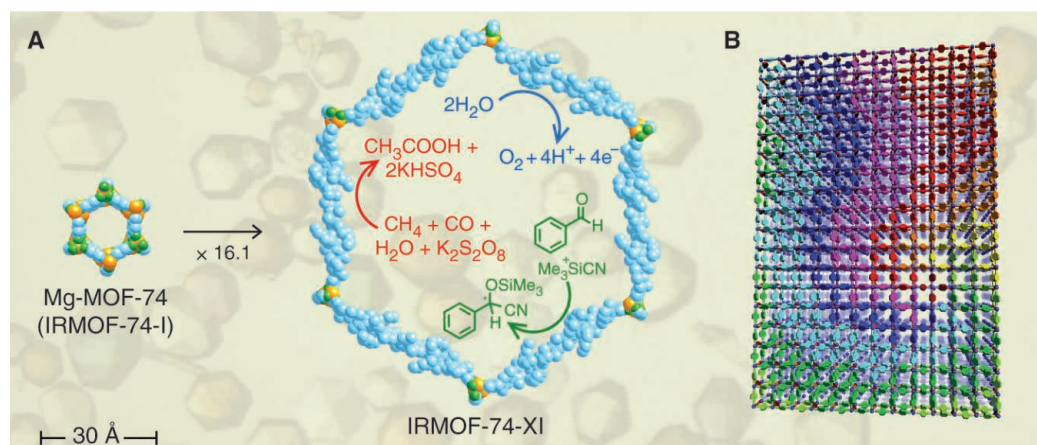
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Background: Metal-organic frameworks (MOFs) are made by linking inorganic and organic units by strong bonds (reticular synthesis). The flexibility with which the constituents’ geometry, size, and functionality can be varied has led to more than 20,000 different MOFs being reported and studied within the past decade. The organic units are ditopic or polytopic organic carboxylates (and other similar negatively charged molecules), which, when linked to metal-containing units, yield architecturally robust crystalline MOF structures with a typical porosity of greater than 50% of the MOF crystal volume. The surface area values of such MOFs typically range from 1000 to 10,000 m²/g, thus exceeding those of traditional porous materials such as zeolites and carbons. To date, MOFs with permanent porosity are more extensive in their variety and multiplicity than any other class of porous materials. These aspects have made MOFs ideal candidates for storage of fuels (hydrogen and methane), capture of carbon dioxide, and catalysis applications, to mention a few.

Advances: The ability to vary the size and nature of MOF structures without changing their underlying topology gave rise to the isoreticular principle and its application in making MOFs with the largest pore aperture (98 Å) and lowest density (0.13 g/cm³). This has allowed for the selective inclusion of large molecules (e.g., vitamin B₁₂) and proteins (e.g., green fluorescent protein) and the exploitation of the pores as reaction vessels. Along these lines, the thermal and chemical stability of many MOFs has made them amenable to postsynthetic covalent organic and metal-complex functionalization. These capabilities enable substantial enhancement of gas storage in MOFs and have led to their extensive study in the catalysis of organic reactions, activation of small molecules (hydrogen, methane, and water), gas separation, biomedical imaging, and proton, electron, and ion conduction. At present, methods are being developed for making nanocrystals and supercrystals of MOFs for their incorporation into devices.

Outlook: The precise control over the assembly of MOFs is expected to propel this field further into new realms of synthetic chemistry in which far more sophisticated materials may be accessed. For example, materials can be envisaged as having (i) compartments linked together to operate separately, yet function synergistically; (ii) dexterity to carry out parallel operations; (iii) ability to count, sort, and code information; and (iv) capability of dynamics with high fidelity. Efforts in this direction are already being undertaken through the introduction of a large number of different functional groups within the pores of MOFs. This yields multivariate frameworks in which the varying arrangement of functionalities gives rise to materials that offer a synergistic combination of properties. Future work will involve the assembly of chemical structures from many different types of building unit, such that the structures’ function is dictated by the heterogeneity of the specific arrangement of their constituents.

Metal-organic framework (MOF) structures are amenable to expansion and incorporation of multiple functional groups within their interiors. (A) The isoreticular expansion of MOFs maintains the network’s topology by using an expanded version of the parent organic linker. Examples of catalysis in MOFs are shown in the large space created by IRMOF-74-XI; Me is a methyl group. (B) Conceptual illustration of a multivariate MOF (MTV-MOF) whose pores are decorated by heterogeneous mixtures of functionalities that arrange in specific sequences. (**Background**) Optical image of zeolitic imidazolate framework (ZIF) crystals.



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ARTICLE OUTLINE

Design of Ultrahigh Porosity

Expansion of Structures by a Factor of 2 to 17

Exceptionally Large Pore Apertures

High Thermal and Chemical Stability

Postsynthetic Modification (PSM):
Crystals as Molecules

Catalytic Transformations Within the Pores

Gas Adsorption for Alternative Fuels and
Separations for Clean Air

Proton Conductivity for Fuel Cell Applications

MOF Nanocrystals

The Materials Beyond

SUPPLEMENTARY MATERIALS

Materials and Methods

Figs. S1 to S8

Tables S1 to S5

References (135–363)

Related Web sites

Cambridge Structural Database reference codes
for MOFs

The Chemistry and Applications of Metal-Organic Frameworks

Hiroyasu Furukawa,^{1,2} Kyle E. Cordova,^{1,2} Michael O’Keeffe,^{3,4} Omar M. Yaghi^{1,2,4*}

Crystalline metal-organic frameworks (MOFs) are formed by reticular synthesis, which creates strong bonds between inorganic and organic units. Careful selection of MOF constituents can yield crystals of ultrahigh porosity and high thermal and chemical stability. These characteristics allow the interior of MOFs to be chemically altered for use in gas separation, gas storage, and catalysis, among other applications. The precision commonly exercised in their chemical modification and the ability to expand their metrics without changing the underlying topology have not been achieved with other solids. MOFs whose chemical composition and shape of building units can be multiply varied within a particular structure already exist and may lead to materials that offer a synergistic combination of properties.

The past decade has seen explosive growth in the preparation, characterization, and study of materials known as metal-organic frameworks (MOFs). These materials are constructed by joining metal-containing units [secondary building units (SBUs)] with organic linkers, using strong bonds (reticular synthesis) to create open crystalline frameworks with permanent porosity (1). The flexibility with which the metal SBUs and organic linkers can be varied has led to thousands of compounds being prepared and studied each year (Figs. 1 and 2). MOFs have exceptional porosity and a wide range of potential uses including gas storage, separations, and catalysis (2). In particular, applications in energy technologies such as fuel cells, supercapacitors, and catalytic conversions have made them objects of extensive study, industrial-scale production, and application (2–4).

Among the many developments made in this field, four were particularly important in advancing the chemistry of MOFs: (i) The geometric principle of construction was realized by the linking of SBUs with rigid shapes such as squares and octahedra, rather than the simpler node-and-spacer construction of earlier coordination networks in which single atoms were linked by ditopic coordinating linkers (1). The SBU approach not only led to the identification of a small number of preferred (“default”) topologies that could be targeted in designed syntheses, but also was central to the achievement of permanent porosity in MOFs (1). (ii) As a natural outcome of the use of SBUs, a large body of work was subsequently reported on the use of the isorecticular principle (varying the size and nature of a structure without changing its underlying topology) in the design

of MOFs with ultrahigh porosity and unusually large pore openings (5). (iii) Postsynthetic modification (PSM) of MOFs—incorporating organic units and metal-organic complexes through reactions with linkers—has emerged as a powerful tool for changing the reactivity of the pores (e.g., creating catalytic sites) (6). (iv) Multivariate MOFs (MTV-MOFs), in which multiple organic functionalities are incorporated within a single framework, have provided many opportunities for designing complexity within the pores of MOFs in a controlled manner (7).

Below, we focus on these aspects of MOF chemistry because they are rarely achieved in oth-

er materials and because they lead to the previously elusive synthesis of solids by design. Unlike other extended solids, MOFs maintain their underlying structure and crystalline order upon expansion of organic linkers and inorganic SBUs, as well as after chemical functionalization, which greatly widens the scope of this chemistry. We review key developments in these areas and discuss the impact of this chemistry on applications such as gas adsorption and storage, catalysis, and proton conduction. We also discuss the concept of MTV-MOFs in relation to the sequence of functionality arrangement that is influenced by the electronic and/or steric interactions among the functionalities. Highly functional synthetic crystalline materials can result from the use of such techniques to create heterogeneity within MOF structures.

Design of Ultrahigh Porosity

During the past century, extensive work was done on crystalline extended structures in which metal ions are joined by organic linkers containing Lewis base-binding atoms such as nitriles and bipyridines (8, 9). Although these are extended crystal structures and not large discrete molecules such as polymers, they were dubbed coordination “polymers”—a term that is still in use today, although we prefer the more descriptive term MOFs, introduced in 1995 (10) and now widely accepted. Because these structures were constructed from long organic linkers, they encompassed void space and therefore were viewed to have the potential to be

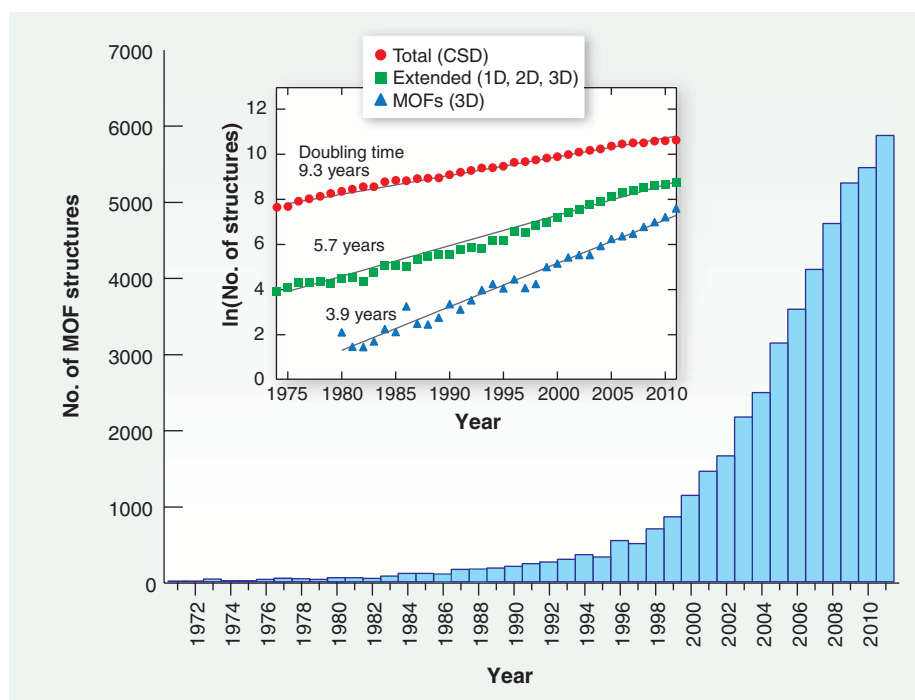


Fig. 1. Metal-organic framework structures (1D, 2D, and 3D) reported in the Cambridge Structural Database (CSD) from 1971 to 2011. The trend shows a striking increase during this period for all structure types. In particular, the doubling time for the number of 3D MOFs (inset) is the highest among all reported metal-organic structures.

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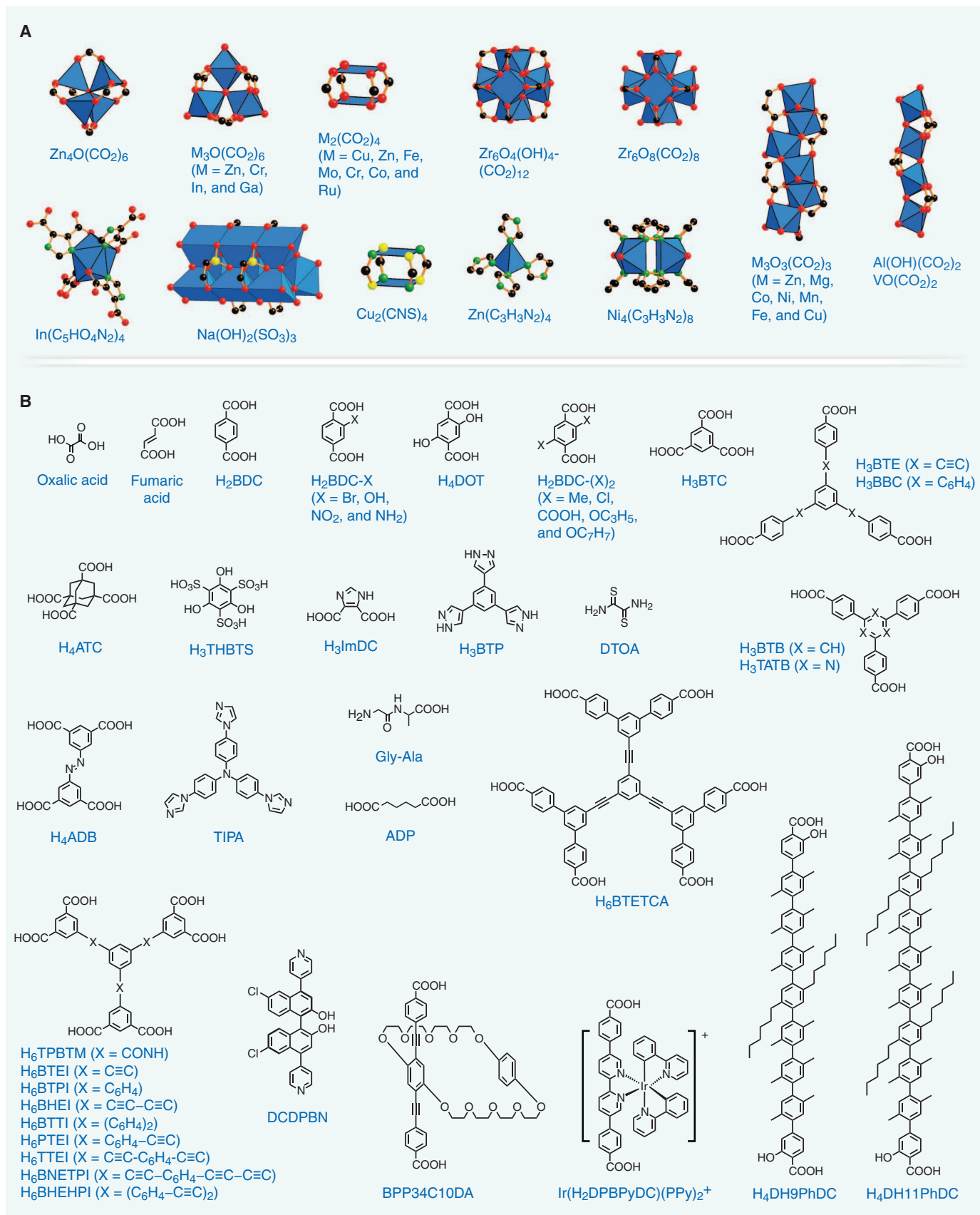


Fig. 2. Inorganic secondary building units (A) and organic linkers (B) referred to in the text. Color code: black, C; red, O; green, N; yellow, S; purple, P; light green, Cl; blue polyhedra, metal ions. Hydrogen atoms are omitted for clarity. AIPA, tris(4-(1H-imidazol-1-yl)phenyl)amine; ADP, adipic acid; TTFTB⁴⁻, 4,4',4'',4'''-([2,2'-bis(1,3-dithiolylidene)]-4,4',5,5'-tetrayl)tetrabenzoate.

permanently porous, as is the case for zeolites. The porosity of these compounds was investigated in the 1990s by forcing gas molecules into the crevices at high pressure (11). However, proof of permanent porosity requires measurement of reversible gas sorption isotherms at low pressures and temperatures. Nonetheless, as we remarked at that time (12), it was then commonplace to refer to materials as “porous” and “open framework” even though such proof was lacking. The first proof of permanent porosity of MOFs was obtained by measurement of nitrogen and carbon dioxide isotherms on layered zinc terephthalate MOF (12).

A major advance in the chemistry of MOFs came in 1999 when the synthesis, x-ray single-crystal structure determination, and low-temperature, low-pressure gas sorption properties were reported for the first robust and highly porous MOF, MOF-5 (13). This archetype solid comprises $\text{Zn}_4\text{O}(\text{CO}_2)_6$ octahedral SBUs each linked by six chelating 1,4-benzenedicarboxylate (BDC^{2-}) units to give a cubic framework (Fig. 2, figs. S2 and S3, and tables S1 and S2). The architectural robustness of MOF-5 allowed for gas sorption measurements, which revealed 61% porosity and a Brunauer-Emmett-Teller (BET) surface area of 2320 m^2/g (2900 m^2/g Langmuir). These values are substantially higher than those commonly found for zeolites and activated carbon (14).

To prepare MOFs with even higher surface area (ultrahigh porosity) requires an increase in storage space per weight of the material. Longer organic linkers provide larger storage space and a greater number of adsorption sites within a given material. However, the large space within the crystal framework makes it prone to form interpenetrating structures (two or more frameworks grow and mutually intertwine together). The most effective way to prevent interpenetration is by making MOFs whose topology inhibits interpenetration because it would require the second framework to have a different topology (15). Additionally, it is important to keep the pore diameter in the micropore range (below 2 nm) by judicious selection of organic linkers in order to maximize the BET surface area of the framework, because it is known that BET surface areas obtained from isotherms are similar to the geometric surface areas derived from the crystal structure (16). In 2004, MOF-177 [$\text{Zn}_4\text{O}(\text{BTB})_2$; $\text{BTB} = 4,4',4''\text{-benzene-1,3,5-triyl-tribenzoate}$] was reported with the highest surface area at that time (BET surface area = 3780 m^2/g , porosity = 83%; Figs. 3A and 4) (15), which satisfies the above requirements. In 2010, the surface area was doubled by MOF-200 and MOF-210 [$\text{Zn}_4\text{O}(\text{BBC})_2$ and ($\text{Zn}_4\text{O})_3(\text{BTE})_4(\text{BPDC})_3$, respectively; $\text{BBC}^{3-} = 4,4',4''\text{-(benzene-1,3,5-triyl-tris(benzene-4,1-diyl))tribenzoate}$; $\text{BTE} = 4,4',4''\text{-(benzene-1,3,5-triyl-tris(ethyne-2,1-diyl))tribenzoate}$; $\text{BPDC} = \text{biphenyl-4,4'-dicarboxylate}$] to produce ultrahigh surface areas (4530 m^2/g and 6240 m^2/g , respectively) and porosities (90% and 89%) (17).

An x-ray diffraction study performed on a single crystal of MOF-5 dosed with nitrogen or argon

gas identified the adsorption sites within the pores (18). The zinc oxide SBU, the faces, and, surprisingly, the edges of the BDC^{2-} linker serve as adsorption sites. This study uncovered the origin of the high porosity and has enabled the design of MOFs with even higher porosities (Fig. 4 and table S3). Moreover, it has been reported that expanded tritopic linkers based on alkyne rather than phenylene units should increase the number of adsorption sites and increase the surface area (19). NU-110 [$\text{Cu}_3(\text{BHEHP})$; $\text{BHEHP}^{6-} = 5,5',5''\text{-(}(((\text{benzene-1,3,5-triyltris(benzene-4,1-diyl))tris(ethyne-2,1-diyl))\text{-tris(benzene-4,1-diyl))tris(ethyne-2,1-diyl))tris(isophthalate)}$], whose organic linker is replete with such edges, displayed a surface area of 7140 m^2/g (Table 1) (7, 17, 20–32).

For many practical purposes, such as storing gases, calculating the surface area per volume is more relevant. By this standard, the value for MOF-5, 2200 m^2/cm^3 , is among the very best reported for MOFs (for comparison, the value for NU-110 is 1600 m^2/cm^3). Note that the external surface area of a nanocube with edges measuring 3 nm would be 2000 m^2/cm^3 . However, nanocrystallites on this scale with “clean” surfaces would immediately aggregate, ultimately leaving their potential high surface area inaccessible.

Expansion of Structures by a Factor of 2 to 17

A family of 16 cubic MOFs—IRMOF-1 [also known as MOF-5, which is the parent MOF of the isorecticular (IR) series] to IRMOF-16—with the same underlying topology (isorecticular) was made with expanded and variously functionalized organic linkers (figs. S2 and S3) (1, 5). This development heralded the potential for expanding and functionalizing MOFs for applications in gas storage and separations. The same work demonstrated that a large number of topologically identical but functionally distinctive structures can be made. Note that the topology of these isorecticular MOFs is typically represented with a three-letter code, **pcu**, which refers to its primitive cubic net (33). One of the smallest isorecticular structures of MOF-5 is $\text{Zn}_4\text{O}(\text{fumarate})_3$ (34); one of the largest is IRMOF-16 [$\text{Zn}_4\text{O}(\text{TPDC})_3$; $\text{TPDC}^{2-} = \text{terphenyl-4,4''-dicarboxylate}$] (5) (fig. S2). In this expansion, the unit cell edge is doubled and its volume is increased by a factor of 8. The degree of interpenetration, and thus the porosity and density of these materials, can be controlled by changing the concentration of reactants, temperature, or other experimental conditions (5).

The concept of the isorecticular expansion is not simply limited to cubic (**pcu**) structures, as illustrated by the expansion of MOF-177 to give MOF-180 [$\text{Zn}_4\text{O}(\text{BTE})_2$] and MOF-200, which use larger triangular organic linkers (**qom** net; Fig. 3A and fig. S4) (15, 17). Contrary to the MOF-5 type of expanded framework, expanded structures of MOF-177 are noninterpenetrating despite the high porosity of these MOFs (89% and 90% for MOF-180 and MOF-200, respectively). These results highlight the critical role of selecting topology.

Another MOF of interest is known as HKUST-1 [$\text{Cu}_3(\text{BTC})_2$; $\text{BTC}^{3-} = \text{benzene-1,3,5-tricarboxylate}$] (35); it is composed of Cu paddlewheel [$\text{Cu}_2(\text{CO}_2)_4$] SBUs (Fig. 2A) and a tritopic organic linker, BTC^{3-} . Several isorecticular structures have been made by expansion with TATB^{3-} [$4,4',4''\text{-(1,3,5-triazine-2,4,6-triyl)tribenzoate}$], TATAB^{3-} [$4,4',4''\text{-(1,3,5-triazine-2,4,6-triyl)tris(azanediyli)tribenzoate}$], TTCA^{3-} [triphenylene-2,6,10-tricarboxylate], HTB^{3-} [$4,4',4''\text{-(1,3,3a',4,6,7,9-heptaazaphenylene-2,5,8-triyl)tribenzoate}$], and BBC^{3-} linkers (**tbo** net; Fig. 3B, fig. S1 and S5, and tables S1 and S2) (21, 36–39). The cell volume for the largest reported member [MOF-399, $\text{Cu}_3(\text{BBC})_2$] is 17.4 times that of HKUST-1. MOF-399 has the highest void fraction (94%) and lowest density (0.126 g/cm^3) of any MOF reported to date (21).

Cu paddlewheel units are also combined with various lengths of hexatopic linkers to form another isorecticular series. The first example of one of these MOFs is $\text{Zn}_3(\text{TPBTM})$ [$\text{TPBTM}^{6-} = 5,5',5''\text{-(benzene-1,3,5-tricarboxyl)tris(azanediyli)tris(isophthalate)}$], which has a **ntt** net (40). Shortly after this report, several isorecticular MOF structures were synthesized (Fig. 3C and fig. S6) (19, 20, 24, 41–48): $\text{Cu}_3(\text{TPBTM})$, $\text{Cu}_3(\text{TDPAT})$, NOTT-112 [$\text{Cu}_3(\text{BTPI})$], NOTT-116 [also known as PCN-68; $\text{Cu}_3(\text{PTEI})$], PCN-61 [$\text{Cu}_3(\text{BTEI})$], PCN-66 [$\text{Cu}_3(\text{NTEI})$], PCN-69 [also known as NOTT-119; $\text{Cu}_3(\text{BTBT})$], PCN-610 [also known as NU-100; $\text{Cu}_3(\text{TTEI})$], NU-108 [$\text{Cu}_3(\text{BTETCA})$], NU-109 [$\text{Cu}_3(\text{BNETPI})$], NU-110, and NU-111 [$\text{Cu}_3(\text{BHEI})$] $\text{TDPAT}^{6-} = 5,5',5''\text{-(1,3,5-triazine-2,4,6-triyl)tris(azanediyli)tris(isophthalate)}$; $\text{BTPI}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(benzene-4,1-diyl))tris(isophthalate)}$; $\text{PTEI}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(benzene-4,1-diyl))tris(ethyne-2,1-diyl))tris(isophthalate)}$; $\text{BTEI}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(ethyne-2,1-diyl))tris(isophthalate)}$; $\text{NTEI}^{6-} = 5,5',5''\text{-(nitrolytris(benzene-4,1-diyl))tris(ethyne-2,1-diyl))tris(isophthalate)}$; $\text{BTBTI}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(biphenyl-4,4'-diyl))tris(isophthalate)}$; $\text{TTEI}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(ethyne-2,1-diyl))tris(benzene-4,1-diyl))tris(ethyne-2,1-diyl))tris(isophthalate)}$; $\text{BTETCA}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(ethyne-2,1-diyl))tris}([(1,1',3',1''\text{-terphenyl})\text{-4,4''-dicarboxylate}])$; $\text{BNETPI}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(ethyne-2,1-diyl))tris(benzene-4,1-diyl))tris(buta-1,3-diyne-4,1-diyl))tris(isophthalate)}$; $\text{BHEI}^{6-} = 5,5',5''\text{-(benzene-1,3,5-triyl-tris(buta-1,3-diyne-4,1-diyl))tris(isophthalate)}$. Isorecticular materials are not necessarily expansions of the original parent MOF, as exemplified by NU-108, because the **ntt** family has a linker (BTETCA^{6-}) with two branching points and two kinds of links (figs. S1B and S7).

A wide variety of metal ions form metal-carboxylate units, and isostructural MOFs can be synthesized by replacing the metal ions in the inorganic SBUs. Indeed, after the appearance of HKUST-1 [$\text{Cu}_3(\text{BTC})_2$], an isostructural series of HKUST-1 [$\text{M}_3(\text{BTC})_2$, where $\text{M} = \text{Zn(II)}$, Fe(II) , Mo(II) , Cr(II) , Ru(II)] was prepared by several groups (fig. S5) (49–53). In the same way as

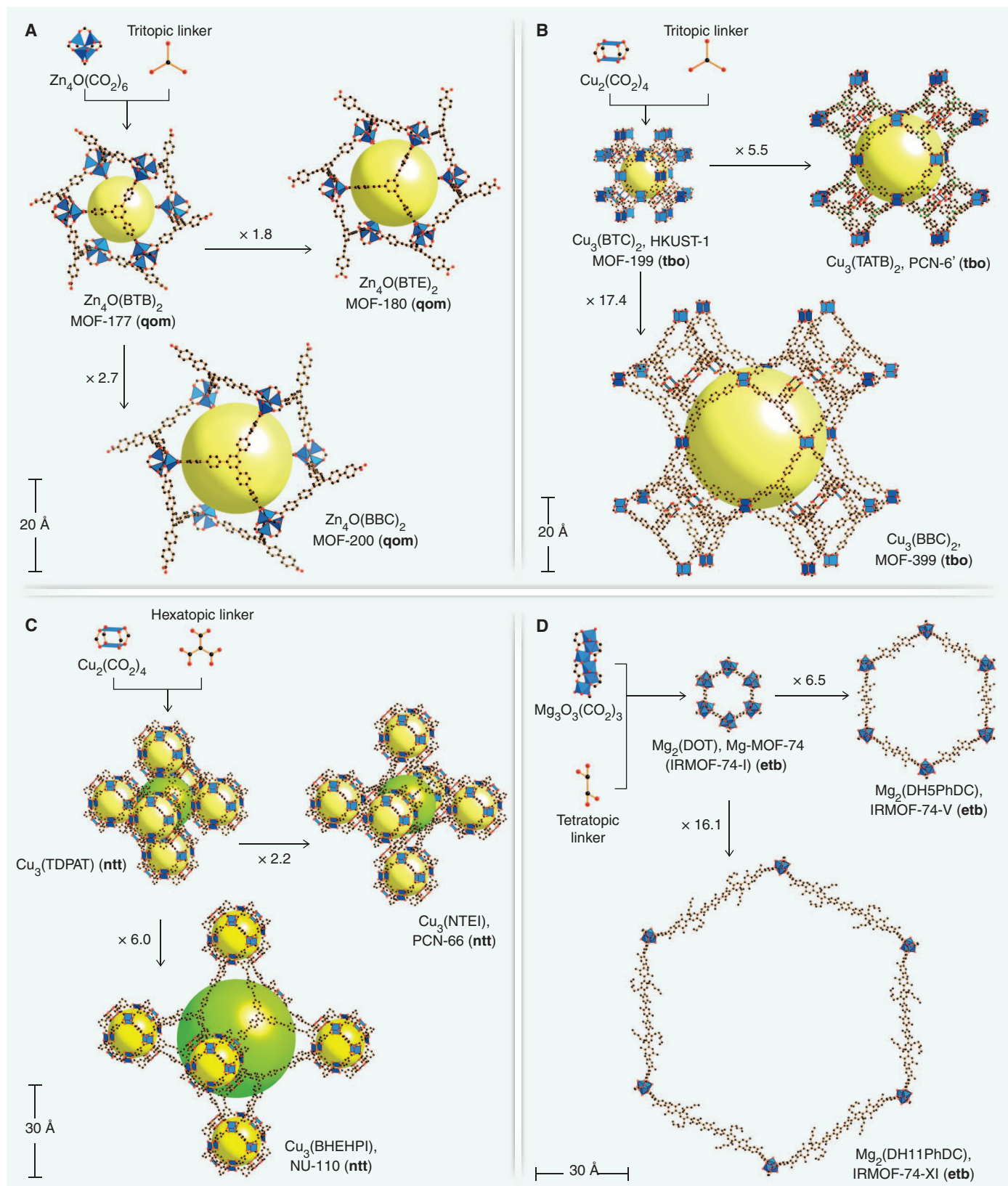


Fig. 3. Isorecticular expansion of metal-organic frameworks with qom, tbo, ntt, and etb nets. (A to D) The isorecticular (maintaining same topology) expansion of archetypical metal-organic frameworks resulting from discrete (A), (B), and (C) and rod inorganic SBUs (D) combined with tri-, hexa-, and tetratopic organic linkers to obtain MOFs in **qom** (A), **tbo** (B), **ntt** (C), and **etb** (D) nets, respectively. Each panel shows a scaled

comparison of the smallest, medium, and largest crystalline structures of MOFs representative of these nets. The large yellow and green spheres represent the largest sphere that would occupy the cavity. Numbers above each arrow represent the degree of volume expansion from the smallest framework. Color code is same as in Fig. 2; hydrogen atoms are omitted for clarity.

discrete inorganic SBUs, the infinite inorganic rod-type SBUs were also used to synthesize isostructural MOF-74 [$\text{Zn}_2(\text{DOT})$; DOT = dioxidoterephthalate] (54) using divalent metal ions such as Mg, Co, Ni, and Mn (fig. S8) (55).

Exceptionally Large Pore Apertures

Pore openings of MOFs are typically large enough (up to 2 nm) to accommodate small molecules,

but rarely are they of appropriate size to permit inclusion of large molecules such as proteins. The best way to increase pore apertures is to use infinite rod-shaped SBUs with linkers of arbitrary length providing periodicity in the other two dimensions, which does not allow for interpenetrating structures. This strategy was implemented by expanding the original phenylene unit of MOF-74 [$\text{M}_2(\text{DOT})$; $\text{M}^{2+} = \text{Zn}, \text{Mg}$] structure (54) to 2, 3,

4, 5, 6, 7, 9, and 11 phenylene units [$\text{DH}_2\text{PhDC}^{4-}$ to $\text{DH}_{11}\text{PhDC}^{4-}$, respectively; Fig. 2B, Fig. 3D, and figs. S1B and S8] (22). Crystal structures revealed that pore apertures for this series of MOF-74 structures (termed IRMOF-74-I to IRMOF-74-XI) ranged from 14 to 98 Å. The presence of the large pore apertures was also confirmed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) observation as well as argon adsorption measurements of the guest-free materials. As expected, the pore aperture of IRMOF-74-IX is of sufficient size to allow for green fluorescent protein (barrel structure with diameter of 34 Å and length of 45 Å) to pass into the pores without unfolding. More important, the large pore aperture is of benefit to the surface modification of the pores with various functionalities without sacrificing the porosity (22). An oligoethylene glycol-functionalized IRMOF-74-VII [$\text{Mg}_2(\text{DH}_7\text{PhDC}-\text{oeg})$] allows inclusion of myoglobin, whereas IRMOF-74-VII with hydrophobic hexyl chains showed a negligible amount of inclusion.

High Thermal and Chemical Stability

Because MOFs are composed entirely of strong bonds (e.g., C-C, C-H, C-O, and M-O), they show high thermal stability ranging from 250° to 500°C (5, 56–58). It has been a challenge to make chemically stable MOFs because of their susceptibility to link-displacement reactions when treated with solvents over extended periods of time (days). The first example of a MOF with exceptional chemical stability is zeolitic imidazolate framework-8 [ZIF-8, $\text{Zn}(\text{MIm})_2$; $\text{MIm}^- = 2\text{-methylimidazolate}$], which was reported in 2006 (56). ZIF-8 is unaltered after immersion in boiling methanol, benzene, and water for up to 7 days, and in concentrated sodium hydroxide at 100°C for 24 hours.

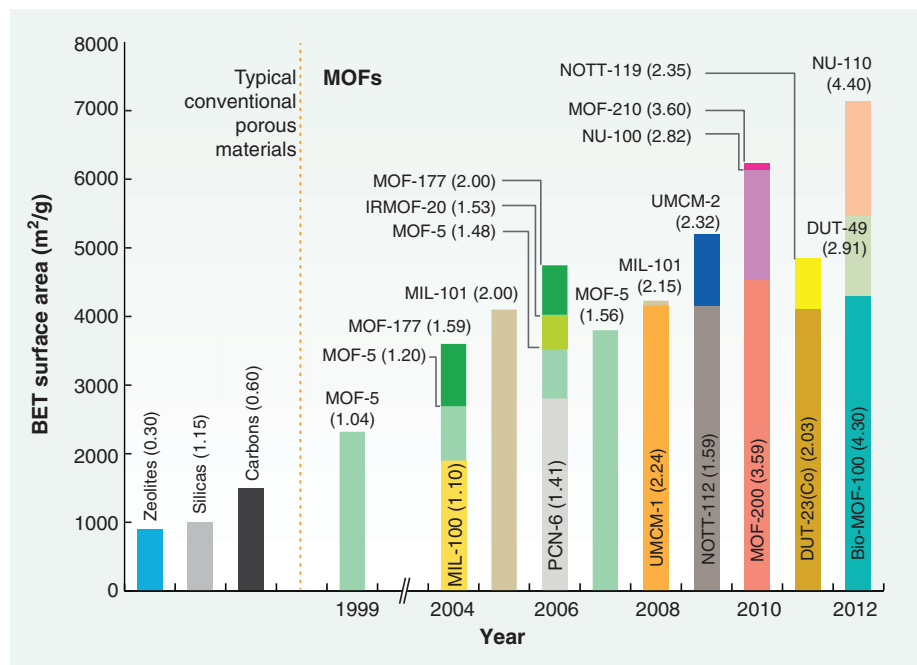


Fig. 4. Progress in the synthesis of ultrahigh-porosity MOFs. BET surface areas of MOFs and typical conventional materials were estimated from gas adsorption measurements. The values in parentheses represent the pore volume (cm^3/g) of these materials.

Table 1. Typical properties and applications of metal-organic frameworks. Metal-organic frameworks exhibiting the lowest and highest values for the indicated property, and those reported first for selected applications, are shown.

Property or application	Compound	Achieved value or year of report	Reference
<i>Lowest reported value</i>			
Density	MOF-399	0.126 g/cm^3	(21)
<i>Highest reported value</i>			
Pore aperture	IRMOF-74-XI	98 Å	(22)
Number of organic linkers	MTV-MOF-5	8	(7)
Degrees of interpenetration	$\text{Ag}_6(\text{OH})_2(\text{H}_2\text{O})_4(\text{TIPA})_5$	54	(23)
BET surface area	NU-110	7140 m^2/g	(20)
Pore volume	NU-110	4.40 cm^3/g	(20)
Excess hydrogen uptake (77 K, 56 bar)	NU-100	9.0 wt%	(24)
Excess methane uptake (290 K, 35 bar)	PCN-14	212 mg/g	(25)
Excess carbon dioxide uptake (298 K, 50 bar)	MOF-200	2347 mg/g	(17)
Proton conductivity (98% relative humidity, 25°C)	$(\text{NH}_4)_2(\text{ADP})[\text{Zn}_2(\text{oxalate})_3] \cdot 3\text{H}_2\text{O}$	$8 \times 10^{-3} \text{ S}/\text{cm}$	(26)
Charge mobility	$\text{Zn}_2(\text{TTFTB})$	0.2 $\text{cm}^2/\text{V} \cdot \text{s}$	(27)
Lithium storage capacity (after 60 cycles)	$\text{Zn}_3(\text{HCOO})_6$	560 mAh/g	(28)
<i>Earliest report</i>			
Catalysis by a MOF	$\text{Cd}(\text{BPy})_2(\text{NO}_3)_2$	1994	(29)
Gas adsorption isotherm and permanent porosity	MOF-2	1998	(12)
Asymmetric catalysis with a homochiral MOF	POST-1	2000	(31)
Production of open metal site	MOF-11	2000	(30)
PSM on the organic linker	POST-1	2000	(31)
Use of a MOF for magnetic resonance imaging	MOF-73	2008	(32)

MOFs based on the Zr(IV) cuboctahedral SBU (Fig. 2A) also show high chemical stability; UiO-66 [$\text{Zr}_6\text{O}_4(\text{OH})_4(\text{BDC})_6$] and its NO_2 - and Br-functionalized derivatives demonstrated high acid (HCl , $\text{pH} = 1$) and base resistance (NaOH , $\text{pH} = 14$) (57, 58). The stability also remains when tetratopic organic linkers are used; both MOF-525 [$\text{Zr}_6\text{O}_4(\text{OH})_4(\text{TpCPP-H}_2)_3$; TpCPP = tetra-*para*-carboxyphenylporphyrin] and 545 [$\text{Zr}_6\text{O}_8(\text{TpCPP-H}_2)_2$] are chemically stable in methanol, water, and acidic conditions for 12 hours (59). Furthermore, a pyrazolate-bridged MOF [$\text{Ni}_3(\text{BTP})_2$; $\text{BTP}^{3-} = 4,4',4''$ -(benzene-1,3,5-triyl)tris(pyrazol-1-ide)] is stable for 2 weeks in a wide range of aqueous solutions ($\text{pH} = 2$ to 14) at 100°C (60). The high chemical stability observed in these MOFs is expected to enhance their performance in the capture of carbon dioxide from humid flue gas and extend MOFs' applications to water-containing processes.

Postsynthetic Modification (PSM): Crystals as Molecules

The first very simple, but far from trivial, example of PSM was with the Cu paddlewheel carboxylate MOF-11 [$\text{Cu}_2(\text{ATC})$; ATC^{4-} = adamantane-1,3,5,7-tetracarboxylate] (30). As-prepared Cu atoms are bonded to four carboxylate O atoms, and the coordination shell is completed typically with coordinated water (Fig. 2A). Subsequent removal of the water from the immobilized Cu atom leaves a coordinatively unsaturated site ("open metal site"). Many other MOFs with such sites have now been generated and have proved to be exceptionally favorable for selective gas uptake and catalysis (61–63).

The first demonstration of PSM on the organic link of a MOF was reported in 2000 for a homochiral MOF, POST-1 [$\text{Zn}_3(\mu_3\text{-O})(\text{D-PTT})_6$; $\text{D-PTT}^- = (4\text{S},5\text{S})$ -2,2-dimethyl-5-(pyridin-4-ylcarbonyl)-1,3-dioxolane-4-carboxylate] (31). It involved *N*-alkylation of dangling pyridyl functionalities with iodomethane and 1-iodohexane to produce *N*-alkylated pyridinium ions exposed to the pore cavity.

More recently, PSM was applied to the dangling amine group of IRMOF-3 [$\text{Zn}_4\text{O}(\text{BDC-NH}_2)_3$] crystals (6). The MOF was submerged in a dichloromethane solution containing acetic anhydride to give the amide derivative in >80% yield. Since then, a large library of organic reactions have been used to covalently functionalize MOF backbones (table S4) (64, 65).

UMCM-1- NH_2 [$\text{Zn}_4\text{O}_3(\text{BDC-NH}_2)_3(\text{BTB})_4$] was also acylated with benzoic anhydride to produce the corresponding amide functionality within the pores (66). The structures of both IRMOF-3 and UMCM-1- NH_2 after modification showed increased hydrogen uptake relative to the parent MOFs, even though there was a reduction in overall surface area (66). PSM has also been used to dangle catalytically active centers within the pores. In an example reported in 2005, a Cd-based MOF built from 6,6'-dichloro-4,4'-di(pyridin-4-yl)-[1,1'-binaphthalene]-2,2'-diol (DCDPBN),

[$\text{CdCl}_2(\text{DCDPBN})$], used orthogonal dihydroxy functionalities to coordinate titanium isopropoxide [$\text{Ti}(\text{O}^i\text{Pr})_4$], thus yielding a highly active, enantioselective asymmetric Lewis acid catalyst (67). UMCM-1- NH_2 was also functionalized in such a manner to incorporate salicylate chelating groups, which were subsequently metallated with Fe(III) and used as a catalyst for Mukaiyama aldol reactions over multiple catalytic cycles without loss of activity or crystallinity (68). Indeed, the remarkable retention of MOF crystallinity and porosity after undergoing the transformation reactions clearly demonstrates the use of MOF crystals as molecules (69).

Catalytic Transformations Within the Pores

The high surface areas, tunable pore metrics, and high density of active sites within the very open structures of MOFs offer many advantages to their use in catalysis (table S5). MOFs can be used to support homogeneous catalysts, stabilize short-lived catalysts, perform size selectivity, and encapsulate catalysts within their pores (70). The first example of catalysis in an extended framework, reported in 1994, involved the cyanosilylation of aldehydes in a Cd-based framework [$\text{Cd}(\text{BPy})_2(\text{NO}_3)_2$; $\text{BPy} = 4,4'$ -bipyridine] as a result of axial ligand removal (29). This study also highlighted the benefits of MOFs as size-selective catalysts by excluding large substrates from the pores.

In 2006, it was shown that removal of solvent from HKUST-1 exposes open metal sites that may act as Lewis acid catalysts (71). MIL-101 [$\text{Cr}_3\text{X}(\text{H}_2\text{O})_2\text{O}(\text{BDC})_3$; $\text{X} = \text{F}, \text{OH}$] and Mn-BTT [$\text{Mn}_3(\text{Mn}_4\text{Cl})_3(\text{BTT})_8$; $\text{BTT}^{3-} = 5,5',5''$ -(benzene-1,3,5-triyl)tris(tetrazol-2-ide)] have also been identified as Lewis acid catalysts in which the metal oxide unit functions as the catalytic site upon ligand removal (62, 72). In addition, alkane oxidation, alkene oxidation, and oxidative coupling reactions have also been reported; they all rely on the metal sites within the SBUs for catalytic activity (73–75). The study of methane oxidation in vanadium-based MOF-48 [$\text{VO}[\text{BDC}(\text{Me})_2]$; $\text{Me} = \text{methyl}$] is promising because the catalytic turnover and yield for this oxidation far exceed those of the analogous homogeneous catalysts (73).

One early example of the use of a MOF as a heterogeneous catalyst is PIZA-3 [$\text{Mn}_2(\text{TpCPP})_2\text{Mn}_3$], which contains a metalloporphyrin as part of the framework (76). PIZA-3 is capable of hydroxylating alkanes and catalyzes the epoxidation of olefins. Schiff-base and binaphthyl metal complexes have also been incorporated into MOFs to achieve olefin epoxidation and diethyl zinc (ZnEt_2) additions to aromatic aldehydes, respectively (67, 77).

The incorporation of porphyrin units within the pores of MOFs can be accomplished during the synthesis (a "ship-in-a-bottle" approach that captures the units as the pores form), as illustrated for the zeolite-like MOF *rho*-ZMOF [$\text{In}(\text{HImDC})_2\text{X}$; $\text{HImDC}^{2-} = \text{imidazoledicarboxylate}$, $\text{X}^- = \text{counteranion}$] (78). The pores of this framework accommodate high porphyrin loadings, and the pore aperture is small enough to prevent porphyrin from

leaching out of the MOF. The porphyrin metal sites were subsequently metallated and used for the oxidation of cyclohexane. The same approach has been applied to several other systems in which polyoxometalates are encapsulated within MIL-101(Cr) and HKUST-1 for applications in the oxidation of alkenes and the hydrolysis of esters in excess water (79, 80).

Integration of nanoparticles for catalysis by PSM has been carried out to enhance particle stability or to produce uniform size distributions. Palladium nanoparticles were incorporated within MIL-101(Cr) for cross-coupling reactions (81, 82). Most recently, a bifunctional catalytic MOF [$\text{Zr}_6\text{O}_4(\text{OH})_4[\text{Ir}(\text{DPBPYDC})(\text{PPy})_2\text{X}]_6$; $\text{DPBPYDC}^{2-} = 4,4'-(2,2'$ -bipyridine)-5,5'-diyl)dibenzoate, $\text{PPy} = 2$ -phenylpyridine] capable of water-splitting reactions was reported (83). This MOF uses the organic linker and an encapsulated nanoparticle to transfer an electron to a proton in solution, leading to hydrogen evolution.

Gas Adsorption for Alternative Fuels and Separations for Clean Air

Much attention is being paid to increasing the storage of fuel gases such as hydrogen and methane under practical conditions. The first study of hydrogen adsorption was reported in 2003 for MOF-5 (84). This study confirmed the potential of MOFs for application to hydrogen adsorption, which has led to the reporting of hydrogen adsorption data for hundreds of MOFs (85). In general, the functionality of organic linkers has little influence on hydrogen adsorption (86), whereas increasing the pore volume and surface area of MOFs markedly enhances the gravimetric hydrogen uptake at 77 K and high pressure, as exemplified by the low-density materials: NU-100 and MOF-210 exhibit hydrogen adsorption as high as 7.9 to 9.0 weight percent (wt%) at 56 bar for both MOFs and 15 wt% at 80 bar for MOF-210 (17, 24). However, increasing the surface area is not always an effective tool for increasing the volumetric hydrogen adsorption, which can be accomplished by increasing the adsorption enthalpy of hydrogen (Q_{st}) (87). In this context, open metal sites have been suggested and used to enhance the hydrogen uptake capacity and to improve Q_{st} (61, 85). Two MOFs with this characteristic, $\text{Zn}_3(\text{BDC})_3[\text{Cu}(\text{Pyen})]$ [$\text{Pyen}^{2-} = 5,5'-(1E,1'E)\text{ethane-1,2-diyl-bis(azanylylidene))bis(methanylylidene))bis(3-methylpyridin-4-ol)}$] and Ni-MOF-74, have the highest reported initial Q_{st} values: 15.1 kJ/mol and 12.9 kJ/mol, respectively (58, 88). Metal impregnation has also been suggested by computation as a method for increasing the Q_{st} values (89). Experiments along these lines show that doping MOFs with alkali metal cations yields only modest enhancements in the total hydrogen uptake and Q_{st} values (90, 91). Although some challenges remain in meeting the U.S. Department of Energy (DOE) system targets (5.5 wt% and 40 g/liter at -40° to 60°C below 100 bar) for hydrogen adsorption (85), Mercedes-Benz has already deployed MOF hydrogen fuel tanks in a

fuel cell–powered demonstration model, the F125 (92).

An alternative high-density fuel source to hydrogen and gasoline is natural gas (methane). The first study of high-pressure methane adsorption in an extended metal-organic structure was reported in 2000 for $\text{CuSiF}_6(\text{BPy})_2$ (93), which demonstrated an uptake capacity of 104 mg/g at 36 atm and 298 K. As is the case for hydrogen adsorption, the total gravimetric methane uptake capacity is generally proportional to the pore volume of MOFs. The calculated total uptake values for MOF-177, MOF-200, and MOF-210 are 345 mg/g, 446 mg/g, and 476 mg/g, respectively, at 80 bar and 298 K. These values are much greater than those of any other MOF (17). The amount of methane stored in a vessel filled with one of these MOFs is at least double the amount that could be stored in an empty vessel at room temperature and pressures up to 80 bar (17). This technology is now being commercialized by BASF for automobile fueling (94). The optimal material is not necessarily the one with highest uptake, but rather one that allows minimal uptake at low pressure and highest uptake at higher pressure so as to maximize the working capacity.

MOFs also offer reversible carbon dioxide adsorption and are promising materials for the selective capture of carbon from the atmosphere and flue gas. Carbon dioxide adsorption in MOFs was first reported in 1998 for MOF-2 [$\text{Zn}(\text{BDC})$] (12). In 2005, a detailed study of carbon dioxide adsorption in a series of MOFs at room temperature showed MOF-177 to have an uptake capacity of 1470 mg/g at 35 bar (95). This uptake of carbon dioxide exceeded that of any known porous material under such conditions. The large quadrupole moment of carbon dioxide molecules causes them to interact with the framework more strongly than hydrogen and methane. As expected, the best excess carbon dioxide uptake reported to date was observed in a MOF with ultrahigh porosity, MOF-200 (2437 mg/g at 50 bar and 298 K) (17). In practical terms, a gas tank filled with MOF-177 or MOF-200 would store 9 times or 17 times as much carbon dioxide at 35 bar, respectively, as the corresponding pressurized tank without MOF. On the other hand, many carbon dioxide capture applications will operate at low pressure so that the Henry's law constant (i.e., initial slope of the isotherm) can be used as an indicator of the carbon dioxide selectivity. MOFs with open metal sites were found to have desirable high initial Q_{st} values of 62 kJ/mol and 47 kJ/mol for MIL-101(Cr) and Mg-MOF-74, respectively (96, 97), thereby offering enhanced carbon dioxide uptake and selectivity at low pressures. Basic nitrogen centers have also been post-synthetically added to MOFs with open metal sites by using N,N' -dimethylethylenediamine (mmen), where the highest initial Q_{st} for $\text{H}_3[(\text{Cu}_4\text{Cl})_3(\text{BTTr})_8(\text{mmen})_{12}]$ [$\text{BTTr}^{3-} = 4,4',4''$ -(benzene-1,3,5-triyl)tris(1,2,3-triazol-1-ide)] is estimated to be 96 kJ/mol (98). Because the ideal material for carbon dioxide capture from flue and combustion

gases requires high selectivity in the presence of water, it is useful to target MOFs in which the competition between carbon dioxide and water for adsorption is minimized. In this respect, chemical binding of carbon dioxide in a recent MOF to make organic carbonates reversibly is a promising approach (99).

Gas storage experiments on MOFs have also been extended to the separation of hydrocarbons, toxic molecules (e.g., ammonia and chlorine), and water. For instance, $\text{Cu}_2(\text{PZDC})_2(\text{Pyz})$ (PZDC = pyrazine-2,3-dicarboxylate; Pyz = pyrazine) selectively takes up acetylene over carbon dioxide through hydrogen bonding between acetylene and oxygen atoms on the MOF internal surface (100). Ammonia was previously considered to be too reactive for MOFs; however, chemically stable Zr-MOFs, such as UiO-66-NH_2 [$\text{Zr}_6\text{O}_4(\text{OH})_4(\text{BDC-NH}_2)_6$] and other derivatives, maintain their structures after the process of ammonia adsorption and desorption (101).

Gas separation processes in MOFs generally rely on both the size of the pores and the affinity of MOFs for the targeted gases. After the discovery of permanent porosity of MOFs, equilibrium adsorption isotherms for various gases were collected to estimate potential gas selectivity. High selectivity calculated from equilibrium data does not guarantee high selectivity under dynamic gas separation conditions. The latter is desirable in industrial processes, because the diffusion rate and Q_{st} value of gas binding in the adsorbent are sensitive to the operating conditions. One of the earliest examples of a dynamic separation was performed using a gas chromatographic column filled with MOF-508 [$\text{Zn}_2(\text{BDC})_2(\text{BPy})$] to separate alkanes such as *n*-pentane, *n*-hexane, 2,2-dimethylbutane, and 2-methylpentane (102). Another early example is the removal of tetrahydrothiophene (THT) from methane by means of a fixed bed containing $\text{Cu}_3(\text{BTC})_2$ (3). The color of the $\text{Cu}_3(\text{BTC})_2$ changed from deep blue to light green, indicating that THT was adsorbed to the copper open metal sites. More recently, it has been reported that Mg and Fe-MOF-74, packed in a column, exhibit enhanced carbon dioxide separation from methane and C_1 to C_3 hydrocarbon, respectively (61, 103).

Proton Conductivity for Fuel Cell Applications

The use of MOFs as inexpensive proton-conducting membranes for fuel cell applications represents a new direction in MOF research (104). The first conductivity measurements were performed on $\text{Cu}(\text{DTOA})(\text{HOC}_2\text{H}_4)_2$, albeit with a relatively low reported conductivity (105). It is now understood that any improvement in the water-mediated proton conductivity of MOFs requires the addition of an acid functionality, such as carboxylic, phosphonic, or sulfonic acid, as demonstrated by several functionalized versions of MIL-53 [$\text{Fe}(\text{OH})(\text{BDC-X})$; $\text{X} = \text{H}, \text{NH}_2, \text{OH}, (\text{COOH})_2$] (106). This finding is further supported by the demonstrated high proton conductivity— 2.5×10^{-3} S/cm at 98% relative humidity and 60°C—of MOFs with highly acidic pores [PCMOF-5, $\text{La}(\text{H}_5\text{DTP})(\text{H}_2\text{O})_3$; $\text{DTP}^{8-} =$

1,2,4,5-tetrakisphosphonomethylbenzene] (107). Alternatively, high proton conductivity is also observed in one-dimensional (1D) metal-organic structures lacking any acidic functionality, as in $\text{Fe}(\text{oxalate})(\text{H}_2\text{O})_2$. Here, coordinated water molecules act as proton donors, with their 1D arrangement within crystals facilitating the rate of proton transport (108).

If MOFs are to serve as proton conductors for practical applications, they must be able to function at relatively high operating temperatures (120° to 180°C) and in anhydrous conditions (109). A simple strategy that may be used to meet these practical considerations is the impregnation of MOFs with amphoteric molecules such as imidazole. For instance, 1*H*-1,2,4-triazole-loaded PCMOF-2 [$\text{Na}_3(\text{THBTS})$; $\text{THBTS}^{3-} = 2,4,6$ -trihydroxy-1,3,5-benzenetrisulfonate] has a higher conductivity than the MOF without triazole (110). Similar results were also obtained for imidazole-doped $\text{Al}(\text{OH})(\text{NDC})$ (NDC = 1,4-naphthalenedicarboxylate) (111). Even higher conductivity was observed when histamine was used as a proton carrier (112). These exciting results indicate that high-mobility proton carriers situated within the pore play a key role in achieving MOFs with high proton conductivity.

Extensive research has been devoted to realizing polymer-supported electrolyte membrane fuel cells consisting of perfluorosulfonic acid polymers (e.g., Nafion); however, these polymers have some drawbacks, such as operation temperature, humidity, and cost (109). MOFs will be attractive candidates for this application because of their tunable pore size and functionality as well as their chemical and thermal stability.

MOF Nanocrystals

The advantages of MOFs are not solely limited to properties that originate from their pore structure and functionalities. By virtue of the diverse synthetic procedures reported, the size and morphology of MOF nanocrystals can be precisely controlled (113). Several groups have recently reviewed the preparation of MOF nanocrystals for membrane and thin-film applications (2, 114, 115).

In 2003, the synthesis of nanocrystalline MOFs was accomplished at room temperature for MOF-5, affording crystal sizes of 70 to 90 nm (116). The obtained MOF samples were crystalline and porous but without controlled morphology. It was not until 2009 that precise control of MOF nanocrystal morphology was reported (117): rhombic dodecahedral ZIF-8 nanocrystals with an estimated average diameter of 40 nm, narrow size distribution, high crystallinity, porosity, and chemical stability. A follow-up study revealed that the nucleation process in ZIF nanocrystal formation is initially slow, followed by a rapid process of crystal growth (118). Interestingly, the study showed that the morphology of the nanocrystals transformed from cubic to rhombic dodecahedra in the last stage of crystal formation. This result indicated that the morphology of nanocrystals can be tuned through the optimization of synthetic conditions. Furthermore, soc-MOF-1

$\{[M_6O_2(ADB)_3(H_2O)_6](H_2O)_6(NO_3)_2; ADB^{4-} = 3,3',5,5'-azobenzene-tetracarboxylate; M = In(III), Ga(III)]\}$ and HKUST-1 nanocrystals have adopted several morphologies with their respective diameters in the submicrometer to micrometer range (119, 120).

The assembly of MOF nanocrystals is of particular interest in MOF research because organized nanostructures comprising one or more nanocrystal types may have distinctive properties similar to those seen in metal nanocrystal superlattices (121). Only a limited number of examples have been reported to date; however, closely packed 2D superlattice structures of ZIF-8, soc-MOF-1, and UiO-66 were observed by SEM (119, 122, 123). Applying an ac electric field created linear chain structures of ZIF-8, and the alignment of the nanocrystals was controlled by the anisotropic nature of surface (124). Clearly, the synthesis of nanocrystals, their assembly into larger structures, and their incorporation into devices are research areas of increasing interest.

The Materials Beyond

At present, MOF chemistry has matured to the point where the composition, structure, functionality, porosity, and metrics of a metal-organic structure can be designed for a specific application. This precise control over the assembly of materials is expected to propel this field further into new realms of synthetic chemistry in which far more sophisticated materials may be accessed. For example, materials can be envisaged as having (i) compartments linked together to operate separately, yet function synergistically; (ii) dexterity to carry out parallel operations; (iii) ability to count, sort, and code information (7); and (iv) capability of dynamics with high fidelity (125, 126).

To appreciate how such materials may be achieved, it is important to step back and consider the gamut of materials studied thus far. In general, crystalline materials are largely composed of a few building units as constituents of their underlying chemical structure. For example, common solid-state materials such as zeolites, alloys, polymers, and even MOFs and nanocrystals are each constructed from one or two kinds of repeating units (Fig. 5). The same is true for the entire landscape of human-made crystalline materials. This self-similarity of structures is quite unlike the heterogeneity and multiplicity that characterize biological molecules. However, the building of materials from as few as three or four different building units remains an outstanding challenge because such chemistry is believed to give mixed phases rather than a single phase comprising mixed components.

It is our view that vast opportunities exist for making materials from an increasing number of unique building units and that a transformative chemistry is yet to be uncovered for achieving these materials. This new materials space that is expected to emerge from linking multiple kinds of building units will have unlimited possibilities, especially when the building units are arranged in specific sequences within crystals. We propose to

call this space “the materials beyond.” One can imagine materials with unique sequences of building units that code for specific functions. Materials capable of complex operations and transformations could be used in applications such as conversion of carbon dioxide to fuels, methane to higher alkanes, and water to hydrogen. Once we begin to think in terms of heterogeneity of composition, shape, size, and orientation of building units, there is no limit to the different scenarios for making materials endowed with unusual complexity. Such materials go beyond merely linking building units through strong bonds, as in MOFs.

In 2010, a simple strategy was proposed and demonstrated to introduce further complexity to crystalline structures (Fig. 6A) (7). In this work, 18 MTV-MOF-5-type structures were synthesized using variously functionalized BDC²⁻ linkers $[NH_2, Br, (Cl)_2, NO_2, (CH_3)_2, C_4H_4, (OC_3H_5)_2, \text{ and } (OC_7H_7)_2]$. One of these structures contains eight different functionalities in one phase. The backbone structure (same as MOF-5) is ordered, whereas functional groups incorporated into the framework are varied and disordered. The complex arrangements of these functionalities in the pores can lead to unusual properties; for instance, MTV-MOFs showed improved hydrogen and carbon dioxide uptake capacities relative to MOFs

comprising a single organic linker (7). This finding clearly demonstrates that MTV-MOFs are not simple linear combinations of their constituents.

Precise control of the functionality arrangement in MOFs is still a looming challenge; however, a new strategy that is widely used for peptide synthesis has been used to systematically control a sequence of functionalities via solid-phase synthesis. Metal-organic complex arrays (MOCAs) are heterometallic complexes in which different metal-organic centers are connected to an amino acid backbone (Fig. 6B) (127). The solid-phase synthesis strategy for connecting these units allowed for careful control of the sequence, nuclearity, and length of up to six metal centers including Pt(II), Rh(III), and Ru(II). Potentially MOCAs can serve as functionalities within MOFs to affect sequence-dependent properties. This idea may also manifest itself in materials recently prepared by the sequential deposition of multilayer MOF thin films (128).

It is not always necessary to exercise multiple linkers or specific sequences to acquire heterogeneity in MOFs without losing either crystallinity or porosity. Recently, microporous MOF-5 materials in which each crystal contains a system of mesopores and macropores have been synthesized by use of a controlled amount of 4-(dodecyloxy)benzoic acid (DBA) to a reaction

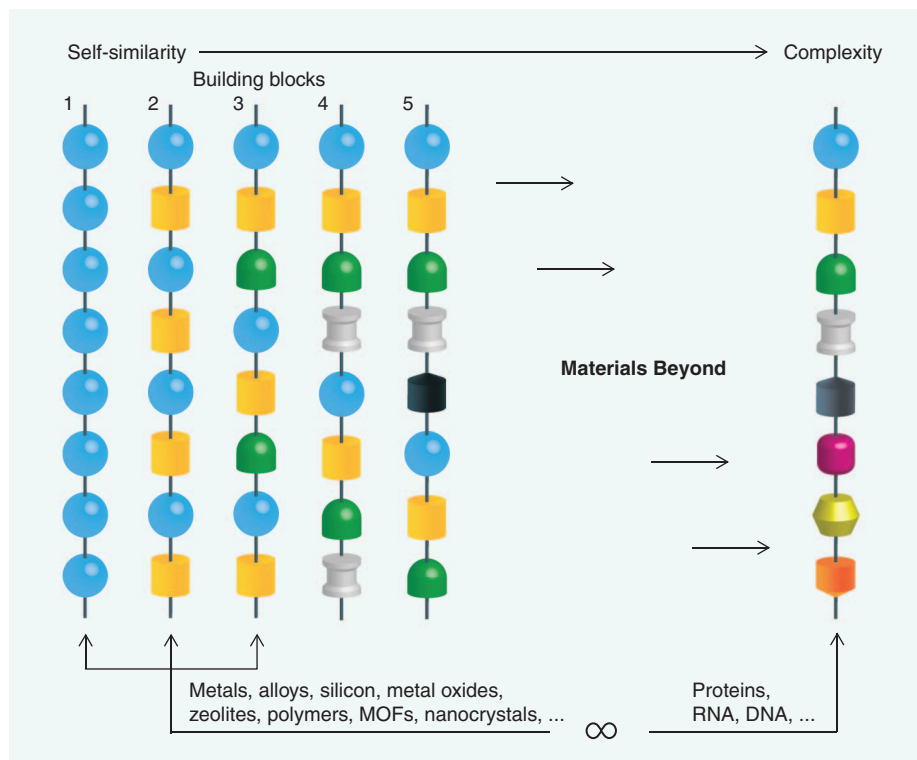


Fig. 5. Crystalline chemical structures of some of the most useful materials. These materials (metals, alloys, silicon, metal oxides, zeolites, and polymers) are constructed from few kinds of building units (one to three) to make largely self-similar materials in which such building units repeat periodically throughout the material. Few examples exist of synthetic crystals with more than three building units, as found in biological structures (proteins, RNA, and DNA). The linking of building units by strong bonds (reticular chemistry) as exemplified by the construction of MOFs is useful in developing the structure and function space of the “materials beyond.”

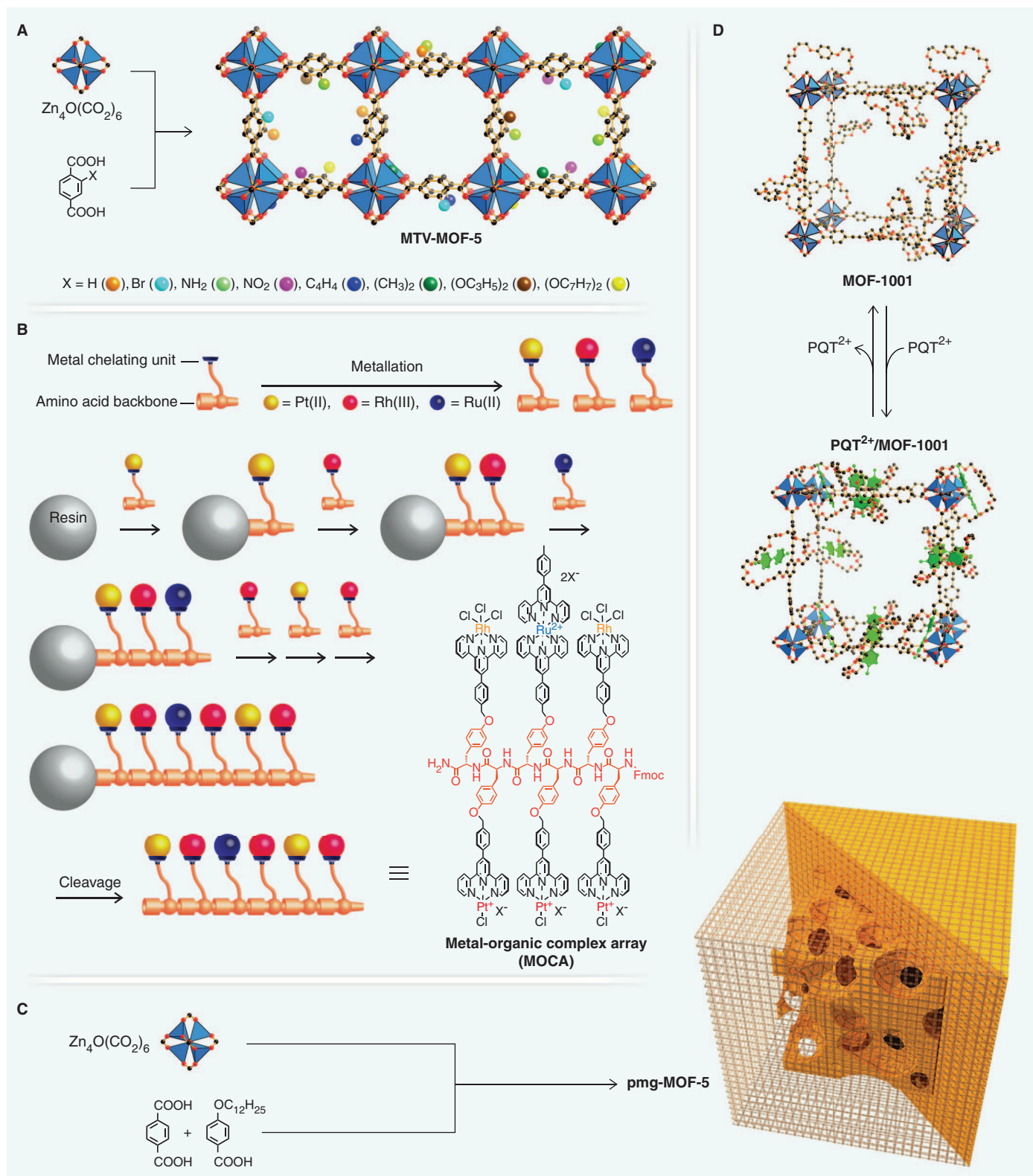


Fig. 6. Examples of creating heterogeneity within crystalline materials. (A) MTV-MOF-5, in which a heterogeneous mixture of functionalities decorates the interior of the crystals to provide an environment capable of highly selective binding of carbon dioxide. (B) Schematic representation of the synthesis of MOCA, which have a heterogeneity created by arranging metal complexes in specific sequences to give sequence-dependent properties. (C)

Heterogeneity within order produced in crystals of MOFs (pmg-MOF-5), where mesopores and macropores are encased in microporous MOF-5 to give unusual carbon dioxide capture properties. (D) A MOF (MOF-1001) constructed from linkers having polyether rings, which in themselves create stereoelectronically specific binding of substrate paraquat (PQT^{2+}). Atom colors are as in Fig. 2; blue polyhedra, Zn. Hydrogen atoms are omitted for clarity.

mixture of MOF-5 (Fig. 6C) (129). By addition of DBA, an entirely sponge-like MOF-5 crystal was obtained, whereas mesopores and macropores fully enclosed by a thick microporous MOF-5 sheath (pomegranate-like crystals; pmg-MOF-5) were obtained when a lesser amount of DBA was used. In situ synchrotron powder x-ray diffraction measurements revealed that the mesopores and macropores of pmg-MOF-5 can act as additional carbon dioxide adsorption sites, even though the sponge-like MOF-5 crystals did not show such behavior.

Heterogeneity can also be introduced when multiple metal ions are used in the synthesis of materials termed core-shell structures (130). Unlike metal-doped MOFs (131), core-shell structures form two compositionally distinct solid phases at their respective interface. Although little is known about the interface of the two phases, film structural analysis of single crystals containing both Zn and Cu domains $\{Zn_2(NDC)_2(DABCO)Cu_2(NDC)_2(DABCO); DABCO = 1,4\text{-diazabicyclo}[2.2.2]\text{octane}\}$ reveals that the Cu phase was grown on the surface of the Zn phase with a small in-plane rotation angle (130). Precise control of such angles may create interfaces that can be exploited for separation and filtration applications.

MOFs comprising interconnected architectural domains that operate independently are also of high interest. In 2009, a “concept transfer” from the biological world successfully accomplished this goal (126). MOF-1001 $[Zn_4O(BPP34C10DA)_3; BPP34C10DA^{2-} = 4,4'-(2,5,8,11,14,16,19,22,25,28\text{-decaoxa-1,15(1,4)-dibenzenacyclooctacosaphane-1}^2,1^5\text{-diylbis(ethyne-2,1-diyl))dibenzoate}]$ uses periodic crown ether receptors attached to the architectural framework; this endows the pore with active domains capable of molecular recognition of highly disordered guests in a stereoelectronically controlled fashion (Fig. 6D) (125). This advancement exploited the mechanism of conformational changes that afford biological macromolecules the capabilities of molecular recognition and installed a new active domain that functions independently of the traditional sorting (i.e., shape and size selectivity of the pore aperture) and coverage domains (i.e., internal pore surface binding). Further evidence for the potential creation of such materials was reported in a MOF $[Zn(Gly-Ala)_2; Gly-Ala = \text{glycylalanine}]$ constructed from a flexible dipeptide linker (132). This MOF displayed adaptable porosity as a result of the linker, evoking comparisons to the conformational selection that is characteristic of proteins.

MOF architectures reminiscent of Russian dolls composed of nested interconnected cages {cage-within-cage structure; CPM-7 $[Zn_{26}O_3(OH)_4(FDA)_{30} \cdot (H_2O)_{12}(Et_2NH_2)_6]$ ($FDA^{2-} = \text{furan-2,5-dicarboxylate}$), CPM-24 $[Co_9(OH)_2(\text{acetate})(BTC)_4(IN)_8 \cdot (H_2O)_4(Me_2NH_2)_5]$ ($IN^- = \text{isonicotinate}$)} can be another way to introduce irregularity within a single parent structure (133, 134). These nested structures provide exciting potential for the assembly of various connected domains whose internal and external

environments can be manipulated to operate independently to suit particular functions (133, 134).

The chemistry and applications of MOFs have progressed substantially since their original inception more than a decade ago. Although it is difficult to rule out the possibility of major advancements arising from MOFs that are compiled from a limited number of building blocks, it is our belief that the future of MOFs lies in the creation of materials whose constituents are many and are systematically varied.

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Supplementary Materials

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Nuclear Lamin-A Scales with Tissue Stiffness and Enhances Matrix-Directed Differentiation

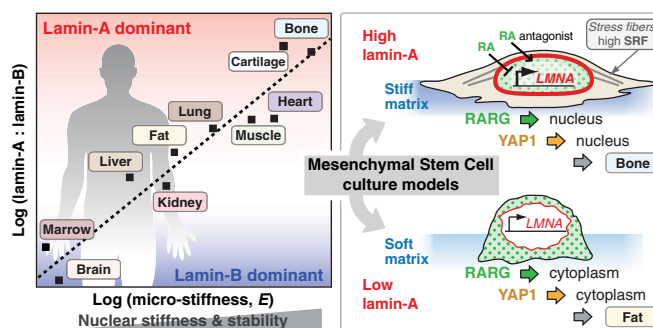
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Introduction: Tissues can be soft like brain, bone marrow, and fat, which bear little mechanical stress, or stiff like muscle, cartilage, and bone, which sustain high levels of stress. Systematic relationships between tissue stiffness, protein abundance, and differential gene expression are unclear. Recent studies of stem cells cultured on matrices of different elasticity, E , have suggested that differentiation is mechanosensitive, but the molecular mechanisms involved in particular tissues remain elusive.

Methods: We developed quantitative mass spectrometry algorithms to measure protein abundance, stoichiometry, conformation, and interactions within tissues and cells in relation to stiffness of tissues and extracellular matrix. Manipulations of lamin-A levels with small interfering RNA, overexpression, and retinoic acid or antagonist were applied to stem cells cultured on different matrices to assess lamin-A's role in mechanosensitive differentiation. To characterize molecular mechanisms, promoter analyses, transcriptional profiling, and localization of transcription factors were complemented by measurements of nuclear mechanics and by modeling of the core gene circuit.

Results: Proteomic profiling of multiple adult solid tissues showed that widely varied levels of collagens in extracellular matrix and of lamin-A in nuclei followed power-law scaling versus E . Scaling for mechanoresponsive lamin-A conformed to predictions from polymer physics, whereas lamin-B's varied weakly. Tumor xenograft studies further demonstrated that matrix determined tissue E , whereas lamin-A levels responded to changes in E . In tissue culture cells, both lamin-A conformation and expression were mechanosensitive, with phosphorylation and turnover of lamin-A correlating inversely with matrix E . Lamin-A knockdown enhanced mesenchymal stem cell differentiation on soft matrix that favored a low-stress, fat phenotype. Lamin-A overexpression or transcriptional induction with a retinoic acid (RA) antagonist enhanced differentiation on stiff matrix toward a high-stress, bone phenotype. Downstream of matrix stiffness, the RA pathway regulated lamin-A transcription, but feedback by lamin-A regulated RA receptor (RARG) translocation into nuclei. High lamin-A levels physically impeded nuclear remodeling under stress but also coregulated other key factors. These factors included both serum response factor (SRF), which promoted expression of stress fiber-associated proteins involved in differentiation, and a Hippo pathway factor (YAP1) involved in growth.

Discussion: The characteristic stress in normal tissue favors collagen accumulation and a characteristic stiffness that cells transduce through nuclear lamin-A to enhance tissue-specific differentiation. Tension-inhibited turnover of rope-like filaments of lamin-A provides sufficient mechanochemical control of a core gene circuit to explain the steady-state scaling of lamin-A with E . High lamin-A physically stabilizes the nucleus against stress and thereby stabilizes the nuclear lamina and chromatin, with implications for epigenetic stabilization and limiting of DNA breaks. Moreover, lamin-A levels directly or indirectly regulate many proteins involved in tissue-specific gene expression, and, because lamin-A levels can vary by a factor of 10 or more downstream of tissue mechanics, an important fraction of tissue-specific gene expression depends on tissue mechanics, which changes in development, injury, and many diseases.



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FIGURES IN THE FULL ARTICLE

Fig. 1. Lamin-A and collagen levels scale with tissue stiffness, but collagen determines stiffness while lamin-A responds.

Fig. 2. Nuclear stability is conferred by lamin-A, which unfolds under stress and which couples to phosphorylation.

Fig. 3. Cell and nuclei spread on stiff matrix, suppressing lamin-A phosphorylation and increasing lamin-A and cell tension.

Fig. 4. Matrix elasticity directs stem cell differentiation, which is enhanced by lamin-A as it regulates SRF and YAP1.

Fig. 5. Matrix stiffness is upstream of RA regulation of lamin-A transcription.

Fig. 6. Lamin-A protein regulates nuclear translocation of RA receptor.

Fig. 7. Lamin-A confers a viscous stiffness to nuclei that impedes nuclear remodeling by stress.

Fig. 8. A feedback-based gene circuit for lamin-A exhibits polymer physics scaling if cell tension suppresses protein turnover.

Box 1. Polymer physics of the nuclear lamina as a shock absorber.

SUPPLEMENTARY MATERIALS

Materials and Methods

Figs. S1 to S16

Tables S1 to S3

References

RELATED ITEMS IN SCIENCE

R. Bainer, V. Weaver, Strength under tension.

Science **341**, 965–966 (2013).

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Tissue micromechanics correlate with abundance of collagens and nuclear lamins, which influence cell differentiation. (Left) Collagen and lamin-A levels scale with E , consistent with matching tissue stress to nuclear mechanics. (Right) Matrix stiffness in tissue culture increases cell tension and stabilizes lamin-A, regulating its own transcription and that of stress fiber genes, enhancing differentiation. RA, retinoic acid, i.e., vitamin A; RARG, YAP1, and SRF, transcription factors.

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Nuclear Lamin-A Scales with Tissue Stiffness and Enhances Matrix-Directed Differentiation

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Tissues can be soft like fat, which bears little stress, or stiff like bone, which sustains high stress, but whether there is a systematic relationship between tissue mechanics and differentiation is unknown. Here, proteomics analyses revealed that levels of the nucleoskeletal protein lamin-A scaled with tissue elasticity, E , as did levels of collagens in the extracellular matrix that determine E . Stem cell differentiation into fat on soft matrix was enhanced by low lamin-A levels, whereas differentiation into bone on stiff matrix was enhanced by high lamin-A levels. Matrix stiffness directly influenced lamin-A protein levels, and, although lamin-A transcription was regulated by the vitamin A/retinoic acid (RA) pathway with broad roles in development, nuclear entry of RA receptors was modulated by lamin-A protein. Tissue stiffness and stress thus increase lamin-A levels, which stabilize the nucleus while also contributing to lineage determination.

Stiffness and strength of a tissue should in principle relate to the physical stress in that tissue. Low stresses in brain and fat may explain why these tissues are soft. High stresses on adult bone, in contrast, are thought to promote its growth and stiffening through a “mechanostat” that functions to match the stress (*1*). At a microscale, physical stress deforms cells (*2*) and can alter gene expression profiles (*3*), but cells in vivo might also directly sense the local tissue stiffness or microelasticity E (in kilopascals, kPa) (table S1), which should relate to the typical stress in that tissue (also in kPa). It is unclear, however, whether any specific proteins function across diverse tissues to not only match stiffness with stress but also impact differentiation processes.

When animal cells are cultured on various gels or elastomeric substrates, cell-generated stress or tension increases as cells spread on matrices with increasing elasticity, E (*4, 5*). Surprising effects on differentiation (*5*), as well as cell shape and motility (*6*), have also been observed. Although some studies have suggested a lack of response to matrix elasticity in two-dimensional (2D) (*7*) or 3D cultures (*8*), several other studies have found that gels that mimic the compliance of brain or fat, respectively, maximize neurogenesis or adipogenesis (*9–11*). Gels that are moderately stiff like muscle are best for myogenesis (*12–14*), and gels that are firm like precalcified bone optimize osteogenesis in 2D and 3D (*5, 15, 16*). A 3D hierarchy of soft/stiff/rigid tissue might exist, but the presence of any molecular mechanostats that relate to tissue stiffness

and that systematically affect lineage remain unknown. Widely expressed transcriptional regulators that include YAP1 of the Hippo pathway, which promotes growth and regeneration (*17*), as well as components of the serum response factor (SRF) pathway, which promote cytoskeletal gene expression in differentiation (*18*), exhibit low nuclear activity in cells on substrates designed to limit cell spreading and cytoskeleton tensions (*11, 19*). How such factors or completely distinct pathways might relate to matrix elasticity and the stiffness of 3D tissues has yet to be addressed.

Forces on a tissue, as well as those generated by cells within a tissue (Fig. 1A), are sustained in rough proportion to microelasticity E by collagens and lineage-specific cytoskeletal proteins (*4, 5*). Some forces might also propagate into the nucleus and be resisted by the nuclear lamina. Lamins are intermediate filament proteins found in nearly all cell nuclei and contribute to nuclear stiffness (*20, 21*) and nuclear stability (*22*). Although lamins might be viewed as similar in mechanical function to keratin intermediate filament proteins that determine nail and skin structure (*23*), lamins are also believed to modulate transcription (*24*) and have been speculated to mechanoregulate the genome (*25, 26*). Here, initial analyses of proteomes from soft and stiff tissues motivated us to examine, both in vivo and in cultures on soft and stiff gels, whether the nuclear lamina is involved in sensing tissue elasticity in differentiation.

Results

Lamin-A and Collagen Levels Scale with Tissue Microelasticity

Allometric scaling laws for stress response would be understandable for polymer-based molecular mechanostats, so we examined proteomes for such trends across tissues from brain to bone

(Fig. 1A). Nearly 100 of the most abundant structural and nuclear proteins were quantified relative to invariant proteins using label-free mass spectrometry (MS) (Fig. 1B and figs. S1 and S2). Lamin-A was found to increase systematically 30-fold from soft to stiff tissue (Fig. 1D and fig. S3). Lamin-B1 differed by less than threefold, and lamin-B2 varied even less (Fig. 1F), consistent with B-type lamins being constitutively expressed (*27*). An absolute stoichiometry of the lamin isoforms (lamin-A:B) was directly determined by MS quantitation of a peptide common to all lamins (see Materials and Methods) (fig. S4, A and B) as validated with recombinant protein (fig. S4C), and a power law fit versus tissue microelasticity gave Lamin-A:B $\sim E^{0.6}$ ($R^2 = 0.88$). Combined with findings that B-type lamins were roughly similar in abundance (fig. S4D), the weak scaling of both B-type lamins is consistent with the key result for Lamin-A versus E as a metric of tissue stress: Lamin-A $\sim E^{0.7}$.

Primary and immortalized cell types derived from a range of human and mouse tissues follow this scaling in terms of E of the tissue of origin, which helps to generalize the result across species and perhaps ameliorate concerns over tissue heterogeneity. Immunoblotting also validated the A:B scaling and further suggested that the A and C splice-form products of the *LMNA* gene follow respective scaling exponents of 1.0 and 0.5, so that the 0.7 exponent for total lamin-A is a geometric mean (Fig. 1E and fig. S5). A power law between concentration of a polymer and its stiffness is typical in the physics of biopolymers (*28–30*). We had previously knocked down lamin-A in human lung-derived A549 cells without affecting lamin-B, and micropipette aspiration showed that knockdown nuclei are softer (*20*), suggesting that nuclear stiffness increases with A:B stoichiometry. Although the tissue E here provides a metric of the typical stress on a tissue, the power law exponent for lamin-A is midway between the linear response of a simple polymer network (*31*) and that of a nonlinear, semiflexible meshwork typified by stiffness versus concentration of actin [with exponent 0.4 (*29*)].

The A:B stoichiometry in Fig. 1D (y axis) indicates that lamin-A dominates over a range of stiff tissues, consistent with *LMNA* mutations causing lipodystrophies, muscular dystrophies, and premature aging (progeria) that affects heart and large vessels while sparing soft tissues such as brain and marrow (*32*). Lamin-B dominates in soft tissues, consistent not only with lamin-A appearing low in antibody-staining of neuroendocrine tissues and hematopoietic cells (*27*) [despite epitope masking (*33*)] but also with lamin-B knockout mice dying at birth with defects in brain development and tissue innervation (*34*). In other words, the normal function of cells in stiff tissues is most dependent on lamin-A.

In nuclear-enriched fractions as well as whole tissue lysates, extracellular matrix proteins were the other detected tissue proteins that scaled

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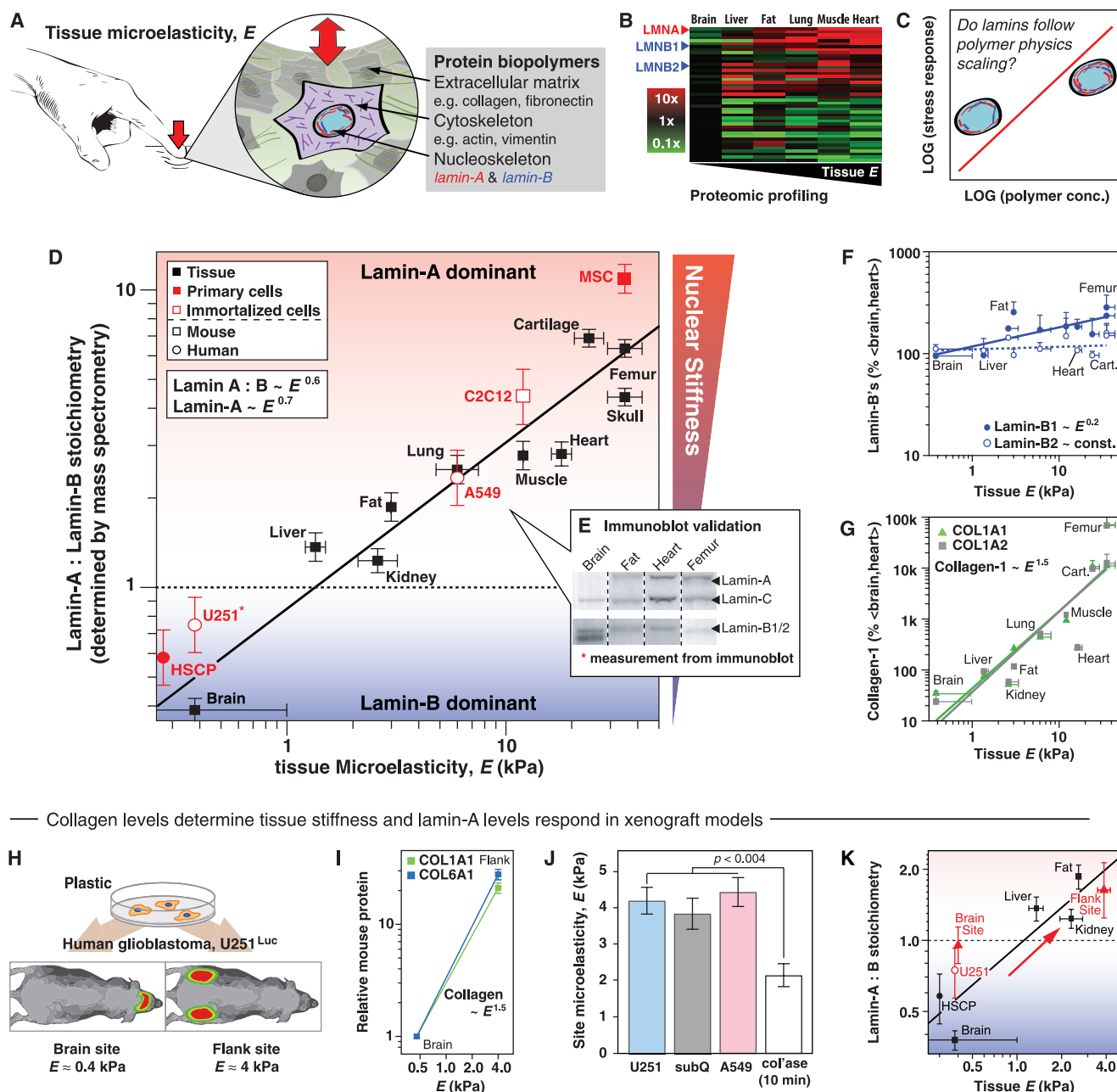
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with E and also showed transcripts scaling with E in both man and mouse (fig. S2). Collagen-1 is the most abundant protein in animals, and its two

fiber-coassembling isoforms both gave collagen-1 $\sim E^{1.5}$ (Fig. 1G). Gels made with purified collagen-1 scale as $\sim E^{0.5}$ (35), but a different

exponent for tissue seems consistent with additional matrix or cell components contributing to tissue mechanics. Indeed, collagen-3, -5, -6, -11,



— Collagen levels determine tissue stiffness and lamin-A levels respond in xenograft models

Fig. 1. Lamin-A and collagen levels scale with tissue stiffness, but collagen determines stiffness while lamin-A responds. (A) Tissue deformation under force is quantified by E and transfers stresses through the extracellular matrix and the cytoskeleton into the nucleus. (B and C) The proteomes of adult mouse tissues were profiled to determine whether scaling of mechanical properties with biopolymer concentration exists across tissues. (D) Quantitative proteomics of multiple human and mouse tissues and cells revealed scaling with E of the absolute ratio or stoichiometry of lamin-A to lamin-B through MS quantification of a pan-lamin peptide. Differences in ratios are significant with brain $\ll$ liver $<$ fat $<$ heart, lung, and muscle $\ll$ skull $\ll$ femur and cartilage, where $<$ indicates $P \leq 0.05$ and $\ll$ indicates $P \leq 0.01$. Nuclei with abundant lamin-A are stiff (20). Cultured cells showed the same trend as their primary source tissue. HSCP, human hematopoietic stem cell progenitors from marrow; U251, human glioblastoma cells from brain; A549,

human adenocarcinoma epithelial cells from lung; C2C12, mouse myoblast cells from muscle; MSC, osteo-prone human mesenchymal stem cells from marrow. (E) MS trends were validated by immunoblotting (representative blots taken from fig. S5A). (F) Lamin-B1 scales very weakly with E , whereas lamin-B2 is constant on average. (G) Collagen-1 isoforms scale strongly with E . (H) Human glioblastoma cells U251^{Luc} (expressing luciferase for imaging) were xenografted into mouse brain and flank, and 4-week-old tumors were profiled by MS proteomics. (I) Mouse-derived collagens in U251 grown in mouse brain and flank scale with E as observed for adult mouse tissues. (J) Stiffness of flank tumors made with high (A549) or low (U251) lamin-A:B cells was similar to the stiffness of the subcutaneous site (subQ). Tumors were 50% softer after only a brief treatment with collagenase (col'ase). (K) Lamin composition and stiffness of the tumors fit adult tissue scaling. All points are significantly different where indicated ($n \geq 3$ MS measurements).

and -12 also scaled as $\sim E^{0.9-1.5}$. Our tissue profiling was unable to identify any compelling cytoskeletal candidate [particularly in the SRF or YAP1 pathways (fig. S1, A to C)] that could be a universal “mechanostat” similar to lamin-A for the nucleus. A possible reason is tissue-specific isoform usage, such as with the intermediate filament protein vimentin, which is restricted to specific lineages rather than being expressed in all cell types. Similar specialization seems likely to apply to isoforms of actin, myosin, and microtubules.

Matrix Determines Tissue Stiffness and Lamin-A Adjusts in Vivo

To address the relative affect of extracellular matrix and lamins on tissue stiffness, human-derived U251 glioblastoma tumors were grown in the brain and in subcutaneous flank sites of nude mice for label-free MS proteomics (Fig. 1H and fig. S6, A to C). In standard culture, these cells had a low A:B ratio similar to normal mouse brain (Fig. 1D). However, flank tumors of U251s had more matrix and were much stiffer than brain tumors, with scaling of collagen density versus E appearing typical of normal adult tissue (Fig. 1I). Flank tumors of human-derived A549 lung cells (A:B $\approx$ 2.3) had similar E as U251 tumors and were only slightly stiffer than normal subcutaneous tissue, revealing a response independent of initial lamin levels (Fig. 1J and fig. S6D). Collagenase treatment of fresh tumors reduced E by $> 50\%$ in just 10 min, suggesting that collagen is a key determinant of tissue stiffness, unlike lamin-A. Consistent with this interpretation, human matrix or matrix-associated proteins were among the few proteins more than twofold higher in the flank compared with the soft brain site. Moreover, human lamin-A levels proved higher in flank versus brain sites (fig. S6), whereas lamin-B1 and lamin-B2 were only slightly higher in brain. U251 cells thus adjust their lamin-A:B ratio by 1.5-fold, which fits remarkably well to the stiffness-dependent scaling of lamin-A:B found in normal tissues (Fig. 1K).

Two other intermediate filament (IF) proteins exhibited site-dependent differences in U251 cells that were notably similar to lamin-A. Human glial fibrillary acidic protein (GFAP) and vimentin were both lower in the softer brain than in the flank (fig. S6B). GFAP expression is known to be restricted to cells of the central nervous system plus a few nonepithelial lineages, so its up-regulation in flank by human brain-derived U251 cells was not expected. Indeed, mouse GFAP was almost undetectable in flank but abundant in brain (fig. S6C). On the other hand, human nestin (yet another IF protein) in the grafted U251 cells was slightly higher in brain than flank, similar in response to the human B-type lamins. These additional findings for lineage-restricted, cytoplasmic IFs thus reinforced the finding that different IFs exhibit different sensitivities to different microenvironments.

Lamin-A Conformation and Abundance Are Mechanosensitive in Cultured Cells

To understand how lamins sustain stress, we focused on cultures of human-derived U251s, A549s,

and low-passage mesenchymal stem cells (MSCs), which collectively span the broad range in lamin-A:B (Fig. 1D). Imaging of lamins under constant immunostaining conditions showed the expected increase in lamin-A intensity as well as juxtaposed networks (24) of lamins (Fig. 2, A and B). To dissect molecular responses of lamins to physical stress, we applied cysteine-shotgun MS (CS-MS) which involves using a fluorescent dye to covalently tag cysteines that are conformationally cryptic but exposed by stress (36). When nuclei were isolated from cells and subjected to controlled shear (Fig. 2C), stability against nuclear rupture was seen to increase with lamin-A levels: $U251 < A549 < [A549 \text{ overexpressing green fluorescent protein (GFP)}\text{-lamin-A}]$ (Fig. 2D). Peeling of lamin-A off of stressed nuclei as seen by immunofluorescence demonstrated the responsiveness of lamin-A to stress. CS-MS revealed several nuclear proteins in the 60 to 80 kD range as susceptible to stress (fig. S7), with Cys⁵²² in lamin-A's immunoglobulin (Ig) domain identified as a stress-sensitive site (Fig. 2E). Studies of pure recombinant Ig domain showed the labeling kinetics of Cys⁵²² captured domain unfolding in thermal and solvent denaturation (Fig. 2F and fig. S8, A to E), and this same site in nuclear lamin-A showed 70% more labeling as shear was increased. A nearby Cys⁵⁹¹ in the tail was also labeled but was insensitive to stress.

A lamin-A point mutation R453W in the Ig domain that causes muscular dystrophy (37) and that destabilized the purified domain (Fig. 2F) also produced dysmorphic nuclei in A549 cells expressing a GFP-lamin-A with the mutation (Fig. 2G). After labeling the adherent cells with the Cys-reactive fluorescent dye, lamin-A was enriched by immunoprecipitation and analyzed by MS (IP-MS). Labeling of the Ig's Cys⁵²² increased significantly (Fig. 2H), whereas labeling of the tail's Cys⁵⁹¹ was unaltered. The mutant also showed fivefold less phosphorylation at a proximal Ser³⁹⁰ without differences at head or tail phospho-Ser (Fig. 2I and fig. S8, F and G); synthetic peptides and phosphopeptides were made and confirmed the linearity of quantitation by MS (fig. S8F). Lamin-A phosphorylation is known to promote disassembly (38) and also protein turnover (39).

Stresses in the cell are transmitted to the nucleus and lamina through various interactions, and because cytoskeletal tension increases with matrix stiffness (5), CS-MS was used to assess matrix effects on lamin-A. MSCs cultured on either soft gels (0.3 kPa) or stiff gels (40 kPa) exhibited the expected low- and high-tension phenotypes with stiffness-induced increases in (i) cell and nuclear spreading (Fig. 3A and fig. S9A), (ii) stress fiber assembly (Fig. 3A and fig. S9, B and C), and (iii) levels of α -smooth muscle actin (Fig. 3, B and C). On soft matrix, the nuclear envelope appeared highly wrinkled (Fig. 3D), but stiff matrix and high tension “smoothed out” nuclear wrinkles and flattened the nucleus. CS-MS was applied to 3-day cultures, with an anticipation of more labeling of the stress-sensitive Ig domain in the high-tension state, but Cys labeling

proved similar in both the Ig and tail sites in cells on soft versus stiff gels (Fig. 3, E and F). On the other hand, phosphorylation proved significantly higher in cells on soft matrices at all four MS-detectable sites (Fig. 3, G and H, and fig. S9D). Because lamin-A phosphorylation promotes disassembly (38) and turnover (39), the results suggested an inverse relationship between phosphorylation and matrix stiffness. Indeed, the total amount of lamin-A increased significantly in both MSCs and A549 cells on stiff substrates (Fig. 3, I and J, and fig. S9, E to G). Lamin-A's increased levels could thus compensate in part for the increased force per molecule in cells on stiff substrates, and this increased level would tend to maintain stability of the protein and its folded domains. Consistent with an increase in IF assembly with cell tension, MSCs treated with a myosin-II inhibitor to inhibit cell tension have also been found to depolymerize vimentin filaments (36). However, because lamin-B did not change significantly with matrix stiffness (fig. S9G) and no lamin-B phosphopeptides were detected, additional study of this mechanism for lamin-A and other mechanosensitive IFs is needed.

Lamin-A Enhances Matrix Elasticity-Directed Differentiation

Matrix elasticity directs lineage specification of human bone marrow-derived MSCs in culture toward bone, fat, or other tissue types with mechanisms based in part on myosin-II generated stresses (5). Because lipodystrophy is one of the many diseases involving *LMNA* and because adipocytes are common in human marrow, the softness of fat was mimicked with a soft gel ($E = 0.3$ kPa), and precalcified bone or “osteoid” was mimicked with a stiff gel ($E = 40$ kPa). Bone marrow-derived MSCs typically have a very high A:B ratio (Fig. 1D) that probably reflects their osteogenic niche origins (40), and indeed even with standard adipogenic media only a very small percentage of MSCs on stiff matrix ($\sim 1\%$) (Fig. 4A) showed after 2 weeks of culture the oil-red-positive lipid droplets that are phenotypic of fat. In these cells, stress fibers were displaced by oil droplets that sometimes deformed the nuclear envelope (Fig. 4B and fig. S10A). Soft matrix increased adipogenesis to 8%, but this increased to nearly 20% with partial knockdown of *LMNA*. MS profiling also revealed an abundant fatty acid ligase (ACSL1) up-regulated nearly 100-fold with knockdown (fig. S11A). Knockdown did not affect adipogenesis of cells on stiff matrix, which invariably showed about 20-fold fewer adipogenic cells than knockdown cells on soft matrix.

Osteogenesis of MSCs was modulated over a 20-fold range through a combination of matrix elasticity and controlled expression of lamin-A (Fig. 4, C and D). Soft matrix always repressed osteogenesis, but stiff matrix plus lamin-A overexpression led to 80% of cells being positive for a standard marker of osteogenesis. Optimal osteogenic conditions also increased endogenous lamin-A expression (by twofold), whereas adipogenic

conditions slightly suppressed lamin-A (Fig. 4E). Thus, insoluble and soluble factors combined to promote lamina remodeling consistent in trend with soft and stiff tissue lineages (Fig. 1D). Although traditional cultures of MSCs on rigid plastic or glass (with unknown matrix) also showed that lamin-A knockdown favored adipogenesis (41) and that lamin-A overexpression favored osteogenesis (42), cultures here on controlled matrix suggest that matrix is upstream, consistent with tissue studies (Fig. 1, H to J).

Matrix elasticity-directed lineage specification of MSCs is based in part on myosin-II-generated cell tension and the accompanying cell spreading (5, 11), which roughly paralleled nuclear shape changes (Figs. 3A and 4E, and fig. S9, A and B). Myosin-IIA was indeed increasingly active and assembled with stiffness-dependent decreases in phosphorylation near myosin-IIA's coiled-coil tail (fig. S9C), as we reported recently (43). Thus, phosphorylation of Ser/Thr residues just beyond the coiled coils (e.g., Fig. 3H) inhibits assembly of

both myosin-IIA and lamin-A homodimers into the respective functional higher-order filaments, and such phosphorylation appeared consistently higher in cells on soft matrices compared with stiff matrices for both proteins. On the other hand, overexpression of lamin-A in cells on stiff matrix did not increase tail phosphorylation of myosin-IIA, suggesting a nonlinear relationship between cytoskeleton tension and lamin-A at the highest levels. Additional indicators of cell tension tended to increase in vitro with matrix elasticity and/or

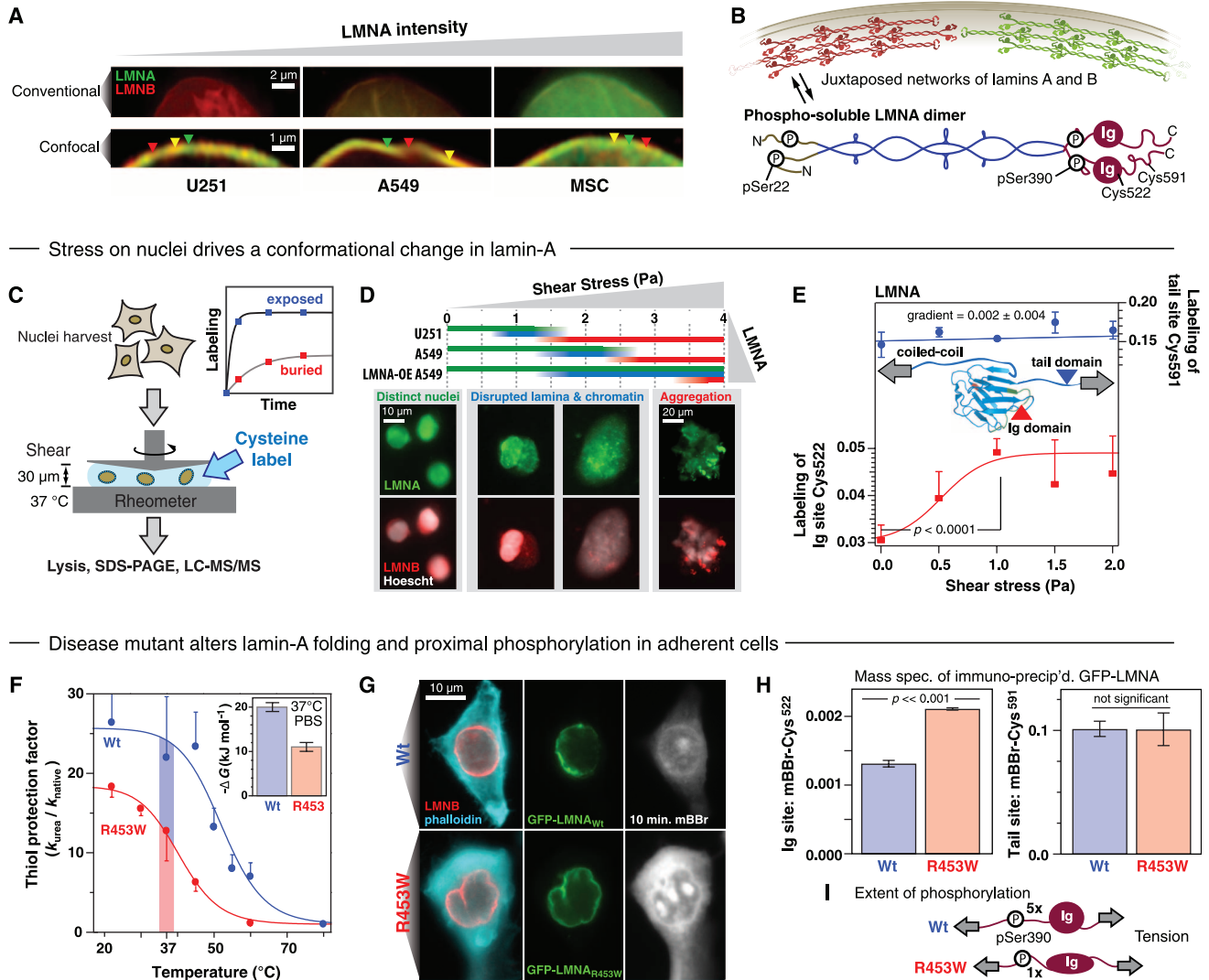


Fig. 2. Nuclear stability is conferred by lamin-A, which unfolds under stress and couples to phosphorylation. (A) High-resolution images of the nuclear envelope of U251s, A549s, and MSCs show juxtapsed regions of lamins A (green) and B (red), consistent with earlier observations in HeLa cells (24). Triangles highlight domains of lamins A (green), B (red), and overlap (yellow). (B) Higher-order assembly of lamin typical of intermediate filament proteins and the lamin-A dimer solubilized by phosphorylation (38), annotated with MS-detectable phosphorylation and cysteine sites. (C) Shearing of nuclei showed that lamin conformation responds to mechanical stress. A cysteine-reactive label [monobromobimane (mBBR)] was added to nuclei and sheared for 40 min at the indicated stresses in a cone and plate rheometer. All protein was then solubilized and the extent of reaction at each detected cysteine quantified by MS, scaled by the unlabeled protein. (D) A549 nuclei imaged following shear stress. Greater lamin expression confers mechanical robustness

to the nuclei, limiting disruption of chromatin. (E) The Ig-like domain of lamin-A has a cryptic cysteine, Cys⁵²², that is buried in the crystal structure (Protein Data Bank accession number 1IFR) but showed 70% more labeling in stressed A549 nuclei. Labeling of Cys⁵⁹¹ in the tail of lamin-A did not change with stress (mean $\pm$ SEM from curve fit; $P \leq 0.05$, $n \geq 3$ MS measurements). (F) A point mutant R453W within the lamin Ig domain that is known to cause muscular dystrophy showed decreased domain stability at 37°C as measured by cysteine labeling rates and tryptophan fluorescence (inset). (G) Images of adherent A549 cells transfected with wild-type or mutant GFP-tagged lamin were labeled with 400 μ M mBBR for 10 min. (H) Labeling of wild-type and R453W lamin of mBBR measured by MS after immunoprecipitation of GFP (IP-MS) showed greater in vivo labeling of mutant in adherent cells; the tail domain showed no significant difference. (I) In contrast, phosphorylation at Ser³⁹⁰ was fivefold higher in wildtype lamin-A. All points are significantly different, as indicated ($n \geq 3$ MS measurements).

lamin-A levels in MSCs. Not only was α -smooth muscle actin suppressed on soft matrix where lamin-A was low (Fig. 3, B and C), but knockdown of lamin-A also suppressed α -smooth muscle actin (*ACTA2*) transcript and protein (Fig. 4, F and G and fig. S12, A and B), together with many other key targets and components of the SRF pathway that regulates expression of *ACTA2* as well as many cytoskeletal genes (18) involved in differentiation to both soft and stiff tissue lineages [neurogenesis (44), myogenesis (18), and osteogenesis (45)]. SRF is regulated in part by nuclear actin (18, 46), and lamin-A binds nuclear actin (47) as well as other proteins that also bind nuclear actin (48); this provided a mechanism for SRF regulation by lamin-A, as also suggested by overexpression studies of one protein (emerin) that binds both lamin-A and actin (49). Tissue analyses showed that SRF target proteins did not generally scale with tissue *E* (figs. S2B and S3D) and that *SRF* and *ACTA2* transcripts increased nontrivially with *E* (fig. S2C). Because high SRF activity can inhibit differentiation of some lineages [e.g., epithelial cells (17)], mechanosensitive lamin-A is likely just one coregulator of the SRF pathway.

One transcription factor implicated in lipodystrophy, SREBP1 (*SREBF1*), is known to bind lamin-A in distributing between nucleus and cytoplasm

(50). SREBP1 is not only an early response factor in adipogenesis (51) but, according to chromatin-IP (fig. S12C), it also regulates *ACSL1* and another adipogenic survival factor *FABP5* (52), both of which increased with LMNA knockdown (Fig. 4F).

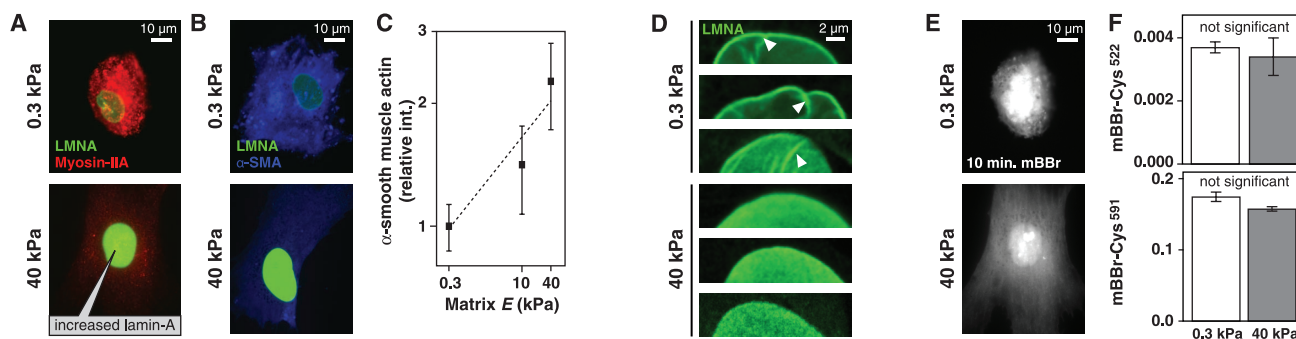
YAP1 has been reported to be excluded from the nucleus in a functionally important manner during adipogenesis of MSCs and also functionally localized to the nucleus during osteogenesis of MSCs (11), but neither *YAP1* transcript levels nor its binding partners or target genes changed with lamin-A knockdown (Fig. 4F). Although YAP1 protein levels did decrease with lamin-A knockdown (fig. S12, A and B), and YAP1 did tend to translocate as expected into the nucleus with increased matrix *E* (Fig. 4, H to J, and fig. S12D), lamin-A overexpression in cells on stiff matrix also produced decreases in both total YAP1 levels and nuclear localization (Fig. 4H). Fluorescence intensity profiles through the nucleus further showed many of the overexpressing cells as well as a fraction of wild-type cells on stiff matrix with YAP1 enriched at the nuclear envelope. The non-monotonic response of YAP1 versus lamin-A levels in vitro was also found for YAP1 protein and transcript levels versus tissue stiffness (Fig. 4K and fig. S12E). Consistent with this, neither YAP1 nor SRF were predicted to directly drive *LMNA* ex-

pression (fig. S13A), and *LMNA* has not been found to be a direct target of these factors as detected by chromatin-IP (18, 53). We thus sought a pathway that could directly regulate *LMNA* and thereby impact lineage.

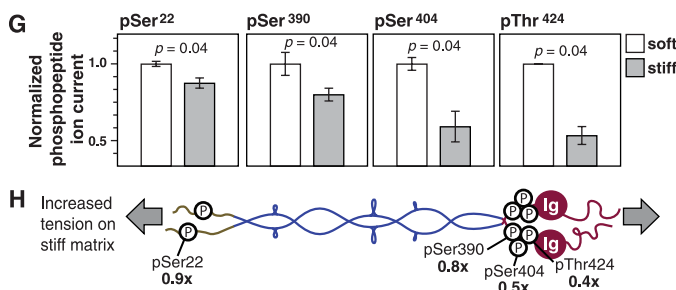
Retinoic Acid Pathway Regulates Lamin-A Transcription, but Lamin-A Protein Regulates an RA Receptor

LMNA level is transcriptionally regulated, with both message and protein fitting the same power law scaling in mouse and man ($R^2 = 0.95$) (Fig. 5A). Promoter methylation was minimal in *LMNA* across a range of cell types (fig. S13B). Bioinformatics analyses of promoters for *LMNA*, *LMNB1*, and *LMNB2* predict retinoic acid (RA) transcription factor sites only in *LMNA* (Fig. 5B and fig. S14), and chromatin-IP has confirmed binding of RA nuclear receptors to *LMNA* (fig. S12C) consistent with experiments on RA-responsive elements (RARE) in *LMNA* (54). Neither RA factors nor collagens were greatly affected by lamin-A knockdown (Fig. 4F and fig. S13C), except for RARB, which is a downstream target of the RA pathway. This placed extracellular matrix upstream of lamin-A together with the level of transcription factors that likely regulate lamin-A, while also suggesting that the RA pathway might be modulated by lamin-A.

Matrix stiffness alters lamina conformation



Matrix stiffness suppresses lamin-A phosphorylation



Matrix stiffness promotes lamin-A

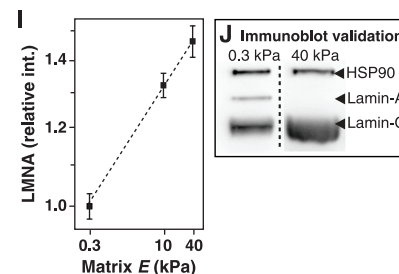
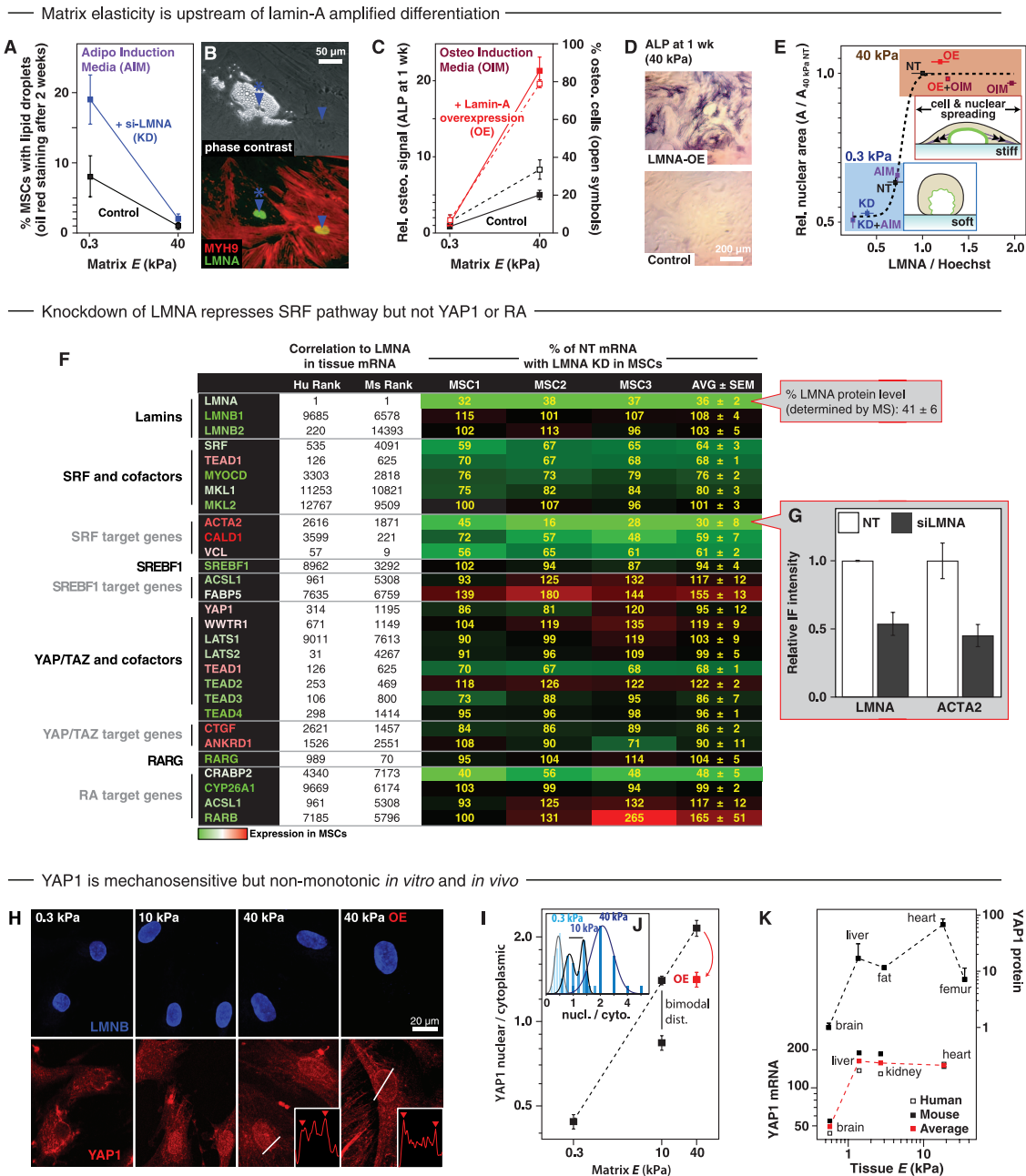


Fig. 3. Cell and nuclei spread on stiff matrix, suppressing lamin-A phosphorylation and increasing lamin-A and cell tension. Response of MSCs to substrate stiffness was characterized. (A) Cells are more rounded on soft (0.3 kPa) matrix, whereas on stiff (40 kPa) matrix they spread with more pronounced stress fibers, consistent with higher cell tension. (B and C) Levels of α -smooth muscle actin were higher on stiff matrix. (D) Confocal microscopy showed wrinkled nuclei on soft matrix, and smoothed-out and flattened nuclei on stiff matrix. Images are of the middle z-section of different nuclei. (E and F)

Cell and nuclei rapidly label with mBBR, but quantitation of lamin-A labeling by IP-MS showed no significant difference in labeling of either the Ig domain or tail sites on soft versus stiff substrate. (G and H) Phosphorylation at Ser³⁹⁰ is ~30% higher on soft substrate, predictive of solubilization. (I and J) Quantitative immunofluorescence and immunoblot show lamin-A increased with substrate stiffness. This tends to reduce the mechanical stress per molecule and maintain the Ig fold. Blots were taken from the same membrane. All points are significantly different ($P \leq 0.05$; $n \geq 3$ MS and IF measurements).

Fig. 4. Matrix elasticity directs stem cell differentiation, which is enhanced by lamin-A as it regulates SRF and YAP1. (A) Partial knockdown (KD) of lamin-A in MSCs with si-LMNA in combination with soft matrix (0.3 kPa) and an adipo-inducing media-maximized adipogenesis ($P \leq 0.02$ knockdown versus control). Stiff matrix (40 kPa) suppressed adipogenesis in parallel cultures, with no significant effect of knockdown. Knockdown of lamin-A was to 35% of wild-type or scrambled-siRNA. (B) Adipogenesis in MSCs on plastic showed that cells with oil droplets (phase contrast microscopy; nucleus indicated by blue arrow with asterisk) had minimal stress fibers (myosin-IIa immunofluorescence) compared with cells without oil droplets (nucleus indicated by blue arrow without asterisk). (C) Overexpression (OE) of lamin-A in MSCs in combination with stiff matrix and an osteo-inducing media-maximized osteogenesis ($P \leq 0.0001$). Soft matrix suppressed osteogenesis in parallel cultures, with no significant effect of overexpression. (D) Alkaline phosphatase (ALP) staining was done after 1 week as a measure of osteogenic signal, together with the fraction of cells with staining. (E) Correlation between nuclear area and lamin-A level with treatments on soft and stiff matrix (normalized to Hoechst stain). NT, non-treated control. Inset cartoons highlight the relationship between cell and nuclear spread area as well as cell tension. (F) Pathway analyses after knockdown of *LMNA* in three different MSCs. Gene symbols are colored according to mRNA abundance in MSCs (green, low; red, high) from microarray data for 11 soft tissues in human and 10 soft tissues in adult mouse (of 14,985 gene annotations common to mouse and human), and genes are ranked based on Pearson correlations with lamin-A. SRF and related transcription factors and target genes all show reduced levels with lamin-A knockdown, whereas neither *YAP1* nor its target genes were affected. *TEAD1* has been implicated in both *YAP1* and SRF pathways, but lamin-A knockdown suppresses *TEAD1* similar to *SRF*, suggesting that it is in the SRF pathway. A transcription factor predicted to regulate lamin-A (*RARG*) was not affected by lamin-A knockdown, and few RA pathway transcripts changed with *LMNA* knockdown except *CRABP2* (89), which decreased. *CRABP2* is up-regulated in osteoarthritis models where *COL1A1* increases in osteogenic-like



processes (90). The SREBF1-regulated gene, *FABP5*, increases to give an average ratio for message of *CRABP2/FABP5* ~ 0.3 relative to untreated cells; both *CRABP2* and *FABP5* are known to bind RA, and the change in the RA signaling ratio (*CRABP2/FABP5*) was consistent with switching of differentiation pathways (91). (G) The decrease in *ACTA2*, downstream of SRF, was confirmed at the protein level in MSCs by immunofluorescence. (H) High-resolution confocal microscopy of YAP1 in MSCs cultured on substrates of increasing stiffness show increasing nuclear localization, as reported previously (11). Insets highlight observation of enrichment at the nuclear envelope, which was especially evident with lamin-A overexpression. (I) Plot shows a fourfold increase in nuclear to cytoplasmic ratio of YAP1 with increasing matrix stiffness in MSCs, except that lamin-A overexpression decreases nuclear YAP1. (J) YAP1 was also bimodally distributed on substrates of intermediate stiffness (10 kPa). (K) YAP1 protein and mRNA levels in tissues of increasing stiffness showed nonmonotonic trends, with the mRNA data averaged from human and mouse microarrays. All points are significantly different, as indicated ($n \geq 3$ imaging, IF, and immunoblot experiments).

Enzymatically derived from vitamin A, RA regulates development and regeneration and is a normal component of serum (~10 nM). It enhances lamin-A expression in embryonic carcinoma cells (54) while repressing lamins in adult granulocyte differentiation (55). Here, a lamin-A promoter driving GFP in A549 cells (Fig. 5C and fig. S14B) showed that RA was repressive, whereas an antagonist (AGN-193109, denoted AGN) enhanced expression (Fig. 5D). RA nuclear receptors are the major RA effectors and were likely involved; indeed, a mutated promoter construct (Δ -LMNA) lacking four of six RAREs (Fig. 5B and fig. S14B)

showed no significant response to RA or AGN (Fig. 5D). Immunoblots of endogenous lamin-A confirmed RA responsiveness (fig. S15, A to E), and a pharmacodynamics study demonstrated nM activity (56) as well as a twofold dynamic range in lamin-A:B expression (fig. S15, F and G). MSCs transfected with the promoter constructs showed an increase in expression in cells on stiff matrix compared with soft matrix for the full promoter, whereas Δ -LMNA showed no significant difference (Fig. 5E). Mechanical and chemical cues were combined to assess lamin-A protein in MSCs cultured on gels (Fig. 5, F and G), with the

cells on stiff matrix (or on plastic) showing the expected lamin-A increase with AGN treatment and decrease with RA, but the effects were entirely suppressed on soft matrix. Matrix elasticity is thus upstream of the RA pathway, which is, in turn, upstream of lamin-A transcription.

Based on RA pathway effects on lamin-A expression in cells on stiff matrix (Fig. 5F), we hypothesized measurable effects on osteogenesis. AGN indeed enhanced osteogenesis of MSCs on stiff matrix, consistent with the AGN-driven increase in lamin-A level, whereas RA suppressed osteogenesis on stiff matrix, and neither drug had

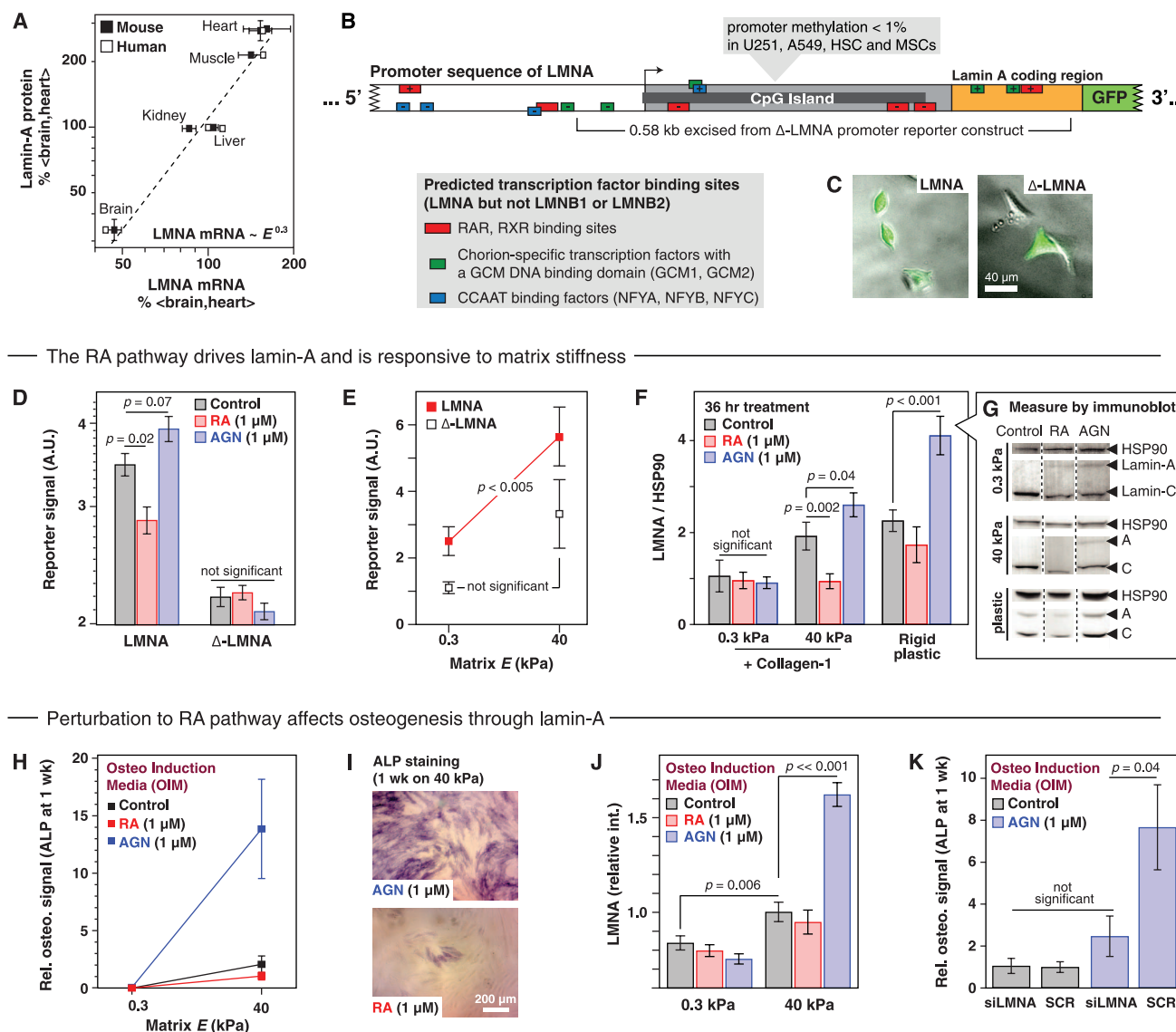


Fig. 5. Matrix stiffness is upstream of RA regulation of lamin-A transcription. (A) LMNA message correlates with protein ($R^2 = 0.95$) across tissue. (B) Promoter-reporter construct for LMNA is annotated with six predicted binding sites of transcription factors in RA pathway and a deletion construct (Δ -LMNA) lacking four RA factor binding sites. (C) A549 cells transfected with GFP reporter constructs. (D) Antagonist (AGN) and agonist (RA) increase and decrease, respectively, expression from the LMNA promoter-reporter and not Δ -LMNA; lamin-A protein shows the same response. (E) LMNA reporter activity increased significantly in MSCs grown on stiff (40 kPa) versus soft (0.3 kPa) matrix, but Δ -LMNA

showed no significant difference. (F) RA and AGN regulate lamin-A in MSCs only on stiff matrix. Gels were coated with collagen-1 for comparison to cultures on untreated plastic, and immunoblotting (G) was performed after 36 hours culture (mean $\pm$ SEM from titration; all blots from same membrane; $P \leq 0.05$; $n \geq 3$ immunoblots). (H) AGN coupled with stiff matrix to increase osteogenesis, determined by ALP staining (I). (J) Increased osteogenic potential was coincident with increased lamin-A levels, measured by immunofluorescence. (K) Lamin-A was necessary for the increased osteogenic potential of MSCs treated with AGN as coincident treatment with siRNA against LMNA-abrogated osteogenesis.

an effect on MSCs on soft matrix (Fig. 5, H and I). The increased osteogenic potential of AGN-treated MSCs on stiff matrix was reflected in higher lamin-A levels (Fig. 5J), and the effect was nullified by simultaneous treatment with small interfering RNA (siRNA) against LMNA (Fig. 5K). Ectopic bone with MSC grafts has been found to be inhibited by RA and a RARG-specific agonist (57, 58), and RARG knockout mice also exhibit osteochondral defects and live only weeks longer than the few weeks that LMNA-deficient mice survive (59–61). Chromatin-IP has thus far identified the RUNX2 gene to be a target of RA transcription factors but not yet SRF (fig. S12C). RARG message generally increased in mouse and man versus tissue *E*, and RARG protein likewise increased in mouse tissue (Fig. 6A and fig. S12E). Fat showed low levels of RARG, and lamin-A knockdown did appear to switch RA pathways toward one that should favor adipogenesis (Fig. 4F). In MSCs, RARG was mostly nuclear in immunostaining (Fig. 6B), and its levels were relatively unperturbed by knockdown of lamin-A (fig. S12, A and B). However, RARG's nuclear-to-cytoplasmic ratio increased fourfold from soft to stiff matrix and was further suppressed by lamin-A knockdown (Fig. 6C). This effect of the lamina on nuclear translocation of RARG was similar in magnitude to the translocation of mechanosensitive YAP1 (Fig. 4I). Moreover, in cells on stiff matrix, RARG also localized to the nuclear envelope (Fig. 6B).

One of the very few other factors found in the nuclear proteome that correlated well with tissue *E* was polymerase I and transcript release factor (PTRF) (fig. S1). PTRF transcript also correlated strongly with both LMNA and COL1A1 across mouse and man transcriptomes (fig. S2D), and lamin-A knockdown strongly decreased PTRF levels in MSCs (fig. S13C). Consistent with PTRF being downstream of lamin-A, chromatin-IP identifies PTRF to be a target gene for both RA and SRF transcription factors (fig. S12C).

To directly perturb the nuclear envelope by means other than knockdown or overexpression of lamin-A, we overexpressed the membrane protein SUN2, which shuttles from the endoplasmic reticulum (ER) to the inner nuclear envelope, where it cross-links the nuclear lamina to the cytoskeleton (48). We hypothesized that SUN2 overexpression would saturate cross-linking sites and effectively decouple the nucleus from the cytoskeleton. SUN2 overexpression indeed produced nuclear rounding and decreased lamin-A levels (figs. S16, A and B), and it also increased cytoplasmic RARG (fig. S16C). SUN2 interacted with lamin-A based on MS analyses of proteins that coimmunoprecipitated with lamin-A (Fig. 6D) [as seen in other assays (62)]. Consistent with such an interaction, nucleus-enriched tissue proteomics indicated $SUN2 \sim E^{0.9}$, even though total SUN2 showed no trend with *E* (fig. S1B). SUN2 was also found in one co-IP with RARG (Fig. 6E), which might explain RARG's enrichment at the envelope (Fig. 6B); however, interactions are likely to be indirect and/or weak

at least because cytoplasmic RARG appeared more diffuse than SUN2 in the ER (fig. S16C). In the same set of experiments, a similar IP-MS approach was taken to identify possible binding partners for YAP1, particularly any factors involved in enrichment at the envelope (Fig. 4H). IP-MS (Fig. 6E) identified the YAP1 paralog TAZ (WWTR1), which has been reported to form a heterodimer with YAP1 (63), and YAP1 phosphorylation at a serine indicated interactions with a key kinase in the Hippo pathway (64). In addition, the co-IP included ELYS (AHCTF1 and MEL-28), which is known to be enriched at the nuclear envelope (65), but any YAP1-ELYS interactions are again likely to be indirect and/or weak and in need of further study together with RARG and SUN2. Nonetheless, our finding that lamin-A protein indirectly regulated lamin-A transcription (through factors that might also bind RARG) means that the apparent mechanoregulation of message could simply be a consequence of feedback from mechanoregulated protein.

High Lamin-A Impedes Nuclear Remodeling Under Stress

Because tissue stress and matrix stiffness stabilize expression of lamin-A, which clearly conferred protection against stress-driven rupture of isolated nuclei (Fig. 2D), we sought a real-time analysis of single-cell nuclear responses to stress. Nuclei in diverse cell types—including embryonic stem cells with very low lamin-A (20)—were aspirated into micropipettes at stresses of kPa, which is similar to tensions in cells (5) and also similar in magnitude to tissue stiffnesses (Fig. 1D). Each nucleus was found to extend in a viscoelastic manner within just ~10 s (Fig. 7, A to C). As a function of lamin-A:B stoichiometry, the effective viscosity increased more rapidly than the effective elasticity (Fig. 7, D and E). Lamin-B thus acted like the elastic walls of a balloon, driving the nucleus to return to its original shape, whereas lamin-A contributed more as a highly viscous fluid within the balloon to impede deformation. Consistent with this physical distinction between lamin isoforms, fluorescence correlation spectroscopy has shown lamin-A to be mobile and lamin-B to be immobile (24).

In vivo cell migration has been seen to dynamically distend a nucleus by twofold or more reversibly over tens of minutes (20). On such long time scales, stress might extend a nucleus locally to a length similar to a typical chromosome (e.g., 5 μ m), but a stiffness-limited extension rate is likely important because elongation of chromatin within the extended nucleus (20) implies rearrangements of chromatin-lamina interactions (66, 67). We hypothesized therefore that across distinct cell types with very different epigenetic features, the time needed for the nucleus to rearrange or relax, τ , would depend primarily on lamin-A level (Fig. 7F). Indeed, for a broad range in lamin-A:B ratio across various glial, epithelial, and mesenchymal cell types with or without knockdown or overexpression of lamin-A, τ varied ~10,000-fold. This level of variation is similar to time-scale differences that would be obtained in aspirating water

versus honey. As functions of lamin-A:B stoichiometry, the steeply positive power laws [$\tau \sim (\text{Lamin-A:B})^{2.5}$] (fig. 7, G and H) were also consistent with predictions from polymer physics (Box 1). The scaling revealed a dominating contribution of lamin-A to nuclear viscosity relative to lamin-B's contribution to nuclear elasticity. While low lamin-A proved insufficient to protect against extreme stresses that completely disrupted chromatin packing (Fig. 2D), the 30-fold higher lamin-A in stiff tissue relative to soft tissue (Fig. 1D) would tend to impede rapid nuclear distension. Thus, high stresses and/or stress fluctuations typical in a stiff tissue such as muscle, heart, or bone (high *E* in Fig. 1D) will not “shock” the nucleus and disrupt chromosome territories or chromatin-lamina interactions (Fig. 7I) that contribute to epigenetic regulation (66, 67) or, in the extreme, cause DNA breaks.

Many progeroid syndromes—beyond progeria due to defective lamin-A—involve mutations that impair proteins important to DNA repair (68). The finding by IP-MS (Fig. 6D) that one DNA repair factor, XRCC6 (Ku70) (69), pulled down with lamin-A from two cell types using high-affinity antibody and that XRCC6 was slightly but consistently decreased upon LMNA knockdown (fig. S13C) suggests a mechanochemical link of DNA repair to lamin-A. Further study is motivated by a previous MS finding that both XRCC6 and ELYS pull down with biotinylated lamin-A bound with low affinity (μ M) to a streptavidin analog (70). Regardless of a possible molecular link to DNA repair factors, lamin-A clearly protects the nucleus against stress.

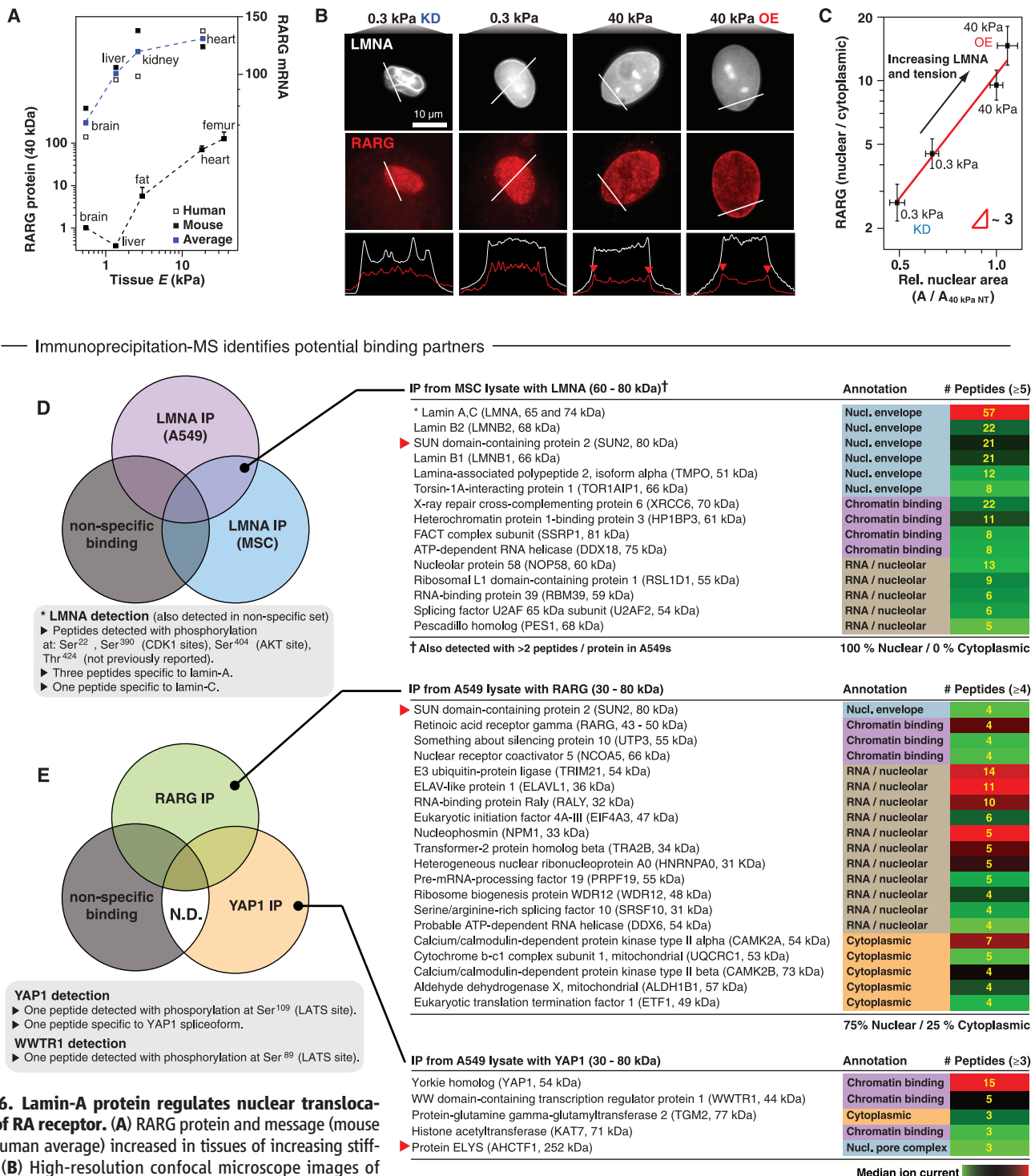
Systems Mechanobiology: Core Gene Circuit Yields Steady-State Scaling for Lamin-A

High matrix stiffness is associated with an increased stress or tension on the nucleus and promotes lamin-A expression and a physically stiffer nucleus. If we thus assume that tension in the rope-like supercoil assemblies of lamin-A filaments suppresses the affinity of an enzyme that initiates phosphorylation/solubilization/degradation of lamin-A, then a parsimonious gene circuit (Fig. 8A) could be modeled mathematically (Fig. 8, B and C), with lamin-A protein effectively feeding back on its own message. Mechanics was explicitly included only in the stress-dependent protein turnover term; synthesis of message and protein as well as degradation of message were all assumed to be linear, with all rate constants chosen to be of order unity. As a test of whether such a model could capture key experimental trends, computational results showed that steady-state lamin-A levels scaled with $(\text{Tension})^{0.7}$, which parallels the scaling of lamin-A with tissue microelasticity *E* (Fig. 1D), noting that $\text{Tension} \sim E$. Kinetic measurements of lamin-A changes with cell mechanical perturbations are clearly needed to further develop such a model.

Discussion

Matrix elasticity is upstream of lamin-A levels in the 3D xenograft model as well as in the 2D

— RARG increases with tissue stiffness and its location in cultured cells is modulated by matrix *E* —



was not present in the sample), were analyzed by MS. **(E)** Proteins associated with immunoprecipitated RARG or YAP1, but not with control samples (a combined list of proteins precipitating with antibody to GFP, not present in the sample, and proteins binding to antibody-free beads). Protein lists were compiled by combining hits from duplicate experiments. The nuclear membrane protein SUN2 was common to both lamin-A and RARG immunoprecipitation experiments. N.D., not detected.

culture models. Lamin-A knockdown in MSCs indeed did not affect expression of the key collagens that scale with tissue stiffness but did suppress the SRF pathway that promotes expression of abundant actin-myosin cytoskeletal components. Soft matrix likewise appears to minimize cytoskeletal stress or tension on the wrinkled nucleus, which thus minimizes stress on lamin-A and thereby favors its phosphorylation and turnover. Low lamin-A protein limits its own transcription by altering nuclear localization of RA transcription factors: Soft matrix and low lamin-A produce the highest cytoplasmic levels of RARG, and the same conditions not only showed the lowest *LMNA* promoter activity but also showed no significant changes of lamin-A protein levels with added RA and AGN. With adipogenic stimuli that include a soft matrix, partial knockdown of

lamin-A in MSCs (to lamin-A:B ~ 3) maximized in vitro adipogenesis, consistent with A:B scaling in tissue profiling. At least for stiff tissue cells with abundant RARG, the response in the vitamin A pathway was also downstream of matrix stiffness. Stiff matrix and high lamin-A led to the highest nuclear levels of RARG, and the same conditions showed not only the highest *LMNA* promoter activity but also about a twofold variation in lamin-A levels upon addition of RA and AGN. AGN enhanced lamin-A, typical of a stiff tissue, and MSC osteogenesis also increased. Differentiation might also be sensitive to splice-forms because AGN increased the A splice-form of *LMNA*, as did forced overexpression, and tissue profiling showed bone has more A than C splice-form, all of which motivates further study. In human bone tissue, lamin-A is among the 20

most abundant proteins detected by MS, together with several SRF-regulated gene products (*ACTB*, *ACTA2*, and *MYL9*) (71), and whereas SRF positively regulates a key transcription factor (*RUNX2*) in osteogenesis (45), chromatin-IP has thus far identified the *RUNX2* gene to be a direct target of RA transcription factors and not of SRF. Lamin-A overexpression in MSCs on stiff matrix not only enhanced osteogenesis but also produced a modest decrease in nuclear YAP1 consistent with the measured nonmonotonic responses of YAP1 across tissues. A complex interplay between YAP1 and/or lamin-A might reconcile past observations that YAP1 promotes osteogenesis (11) but also inhibits *RUNX2* (72); switching between YAP1 and TAZ (*WWTR1*) activities is also possible, because TAZ can promote osteoblast differentiation of MSCs by enhancing *RUNX2*-dependent transcriptional

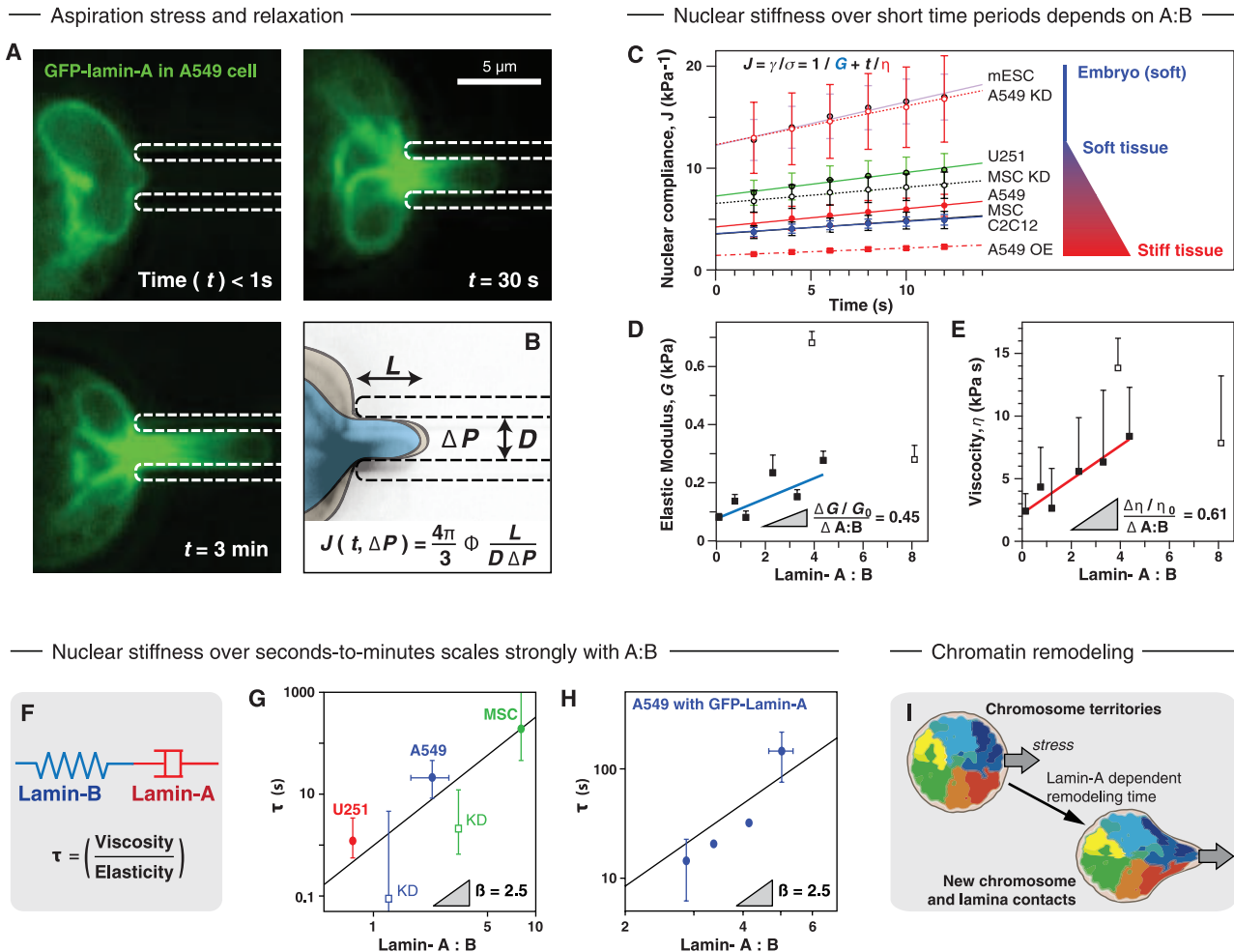


Fig. 7. Lamin-A confers a viscous stiffness to nuclei that impedes nuclear remodeling by stress. (A) Micropipette aspiration of an A549 cell nucleus expressing GFP-lamin-A shows extension of the lamina with time. (B) Schematic showing how nuclear compliance is calculated from image analysis as a function of time and aspiration pressure. (C) Modeling compliance over the first 12 s of deformation, with contributions from elasticity (G) and viscosity (η) in nuclei with different lamina compositions. (D and E) Relationship between the characteristic lamin-A:B ratio and (D) the elastic modulus or (E) the viscosity. The outlier points, A549 OE and MSC, indicated by open symbols, were omitted from the linear fits. (F) The response of the lamina can be

considered as a combination of elastic and viscous components, with an elongation response time, τ (see Box 1). τ was calculated for nuclei extended to ~5 μm by micropipette aspiration over seconds-to-minutes time scales in cells with different lamin-A:B ratios (G) and in A549 cells overexpressing GFP-lamin-A (H). Lamin ratios were calculated from a combination of immunoblotting and MS methods. The scaling of τ with changes in the lamin-A:B ratio, β, was found to be the same in both experiments. (I) A potential biological consequence of nuclear distension is the remodeling of chromosome territories and chromatin-envelope interactions. All points are significantly different where indicated (n ≥ 3 nuclei).

activation (73), and the IP-MS data suggested a YAP1-TAZ interaction. Regardless, the proposed gene circuit for lamin-A seems to be one important core module that modulates various transcriptional pathways (RA, SREBP1, SRF, and YAP1) in enhancing matrix elasticity-directed differentiation.

Our multifaceted approach to a broad range of solid tissues using proteomics and transcriptomics plus knockdown in a highly plastic stem cell that expresses abundant lamin-A seems useful for generic pathway analyses. Indeed, polymerase I and transcript release factor (PTRF) was one of the few factors that not only correlated well with tissue *E*, and decreased with lamin-A knockdown, but also is a known target of RA and SRF transcription factors. The additional fact that mutations in human *PTRF* cause muscular dystrophies and lipodystrophies that also result from *LMNA* mutations (74) suggests an additional feedback into intersecting pathways. Understanding the physiological determinants of normal lamin levels could thus begin to clarify why some laminopathies have phenotypes that dominate in a particular subset of tissues.

Beyond a role in regulating transcription factors, the lamins interact directly with many other nuclear proteins and form lamina-associated domains (LADs) that are repressive regions rich in heterochromatin (66, 67). LADs have roles in some aspects of lineage-specific differentiation, and so the 30-fold variations in lamin-A with tissue stiffness imply a physical regulation of LADs. The relative abundance of A versus B-type lamins could be critical, and despite the weak scaling observed for lamin-B1 and -B2 in solid tissues,

nucleated blood cells show large variations in lamin-B (55). Mechanisms of regulation of lamin-Bs within soft marrow are thus far unclear, but human hematopoietic stem cells (HSCs) do exhibit a soft nuclear phenotype (20) consistent with soft marrow. The MSCs studied herein were also human bone marrow-derived, and their potential to contribute to both rigid bone and softer marrow fat is relevant to marrow microenvironments and to a distinct influence of osteoblasts and adipocytes on HSCs (75).

Lamins as Stress-Modulated Lineage Enhancers

Neither A- nor B-type lamins are essential for lineage induction or specification. *LMNA* knock-out mice develop all tissues but die weeks after birth with growth retardation of connective tissues and also muscular dystrophy (59–61) that is very severe compared with the prototypic mouse model of muscular dystrophy (*Dmd^{mdx}*), which lives for 2 years (76). *LMNA* expression is therefore essential for maturation and survival against the incessant stressing in adult tissues, and once the lamin level matches to the stress, then the optimal level can enhance a prespecified lineage. The weakly modulated B-type lamins are also dispensable in embryonic development; lamin-B knockout mice die at birth as neurons apoptose (34) in migration through the dense midcortex, which stretches normal nuclei by four- to fivefold (20). The lamin knockouts thus suggest important structure-stabilizing roles for the lamins, but the findings reveal lamin-A to be the most stress-inducible differentiation-enhancing factor and lamin-B2 to be the most refractory. Distinct reg-

ulation of stress-inducible isoforms versus constitutive isoforms is reminiscent of the heat shock protein 90 isoform family that constitutes roughly 2% of protein mass in mammalian cells (77). This seems no coincidence because mechanical work and heat are, after all, two key terms in inescapable thermodynamic laws that cells have evolved and adapted to.

Although softening or stiffening the nucleus through lamin-A knockdown or overexpression could physically modulate the cytoskeleton and cell spreading in a manner similar to matrix elasticity, nuclear effects were not as large as matrix effects. This could be because cytoskeleton-nuclear interactions are several times smaller in net area than cell-matrix interactions; from this purely physical point of view, matrix elasticity should be upstream of nuclear mechanics and lamin regulation. In addition, tissue elasticity does not appear greatly affected by nuclear mechanics: In a stiff tissue such as skeletal muscle (*E* ~ 12 kPa), nuclei make up just 0.15% of muscle volume, whereas cytoskeletal volume is almost 60% (78), and in a much softer tissue such as liver (*E* ~ 1.5 kPa), the nuclear-to-cytoplasmic percentage is much higher but still small at around 6 to 7% (79). Nonetheless, lamin-A:B levels did adjust by about twofold in primary adult cells (MSCs) as well as cell lines (U251 and A549) in response to tissue microenvironments in vivo, or else soft gels and stiff substrates (including rigid plastic), and/or soluble factors in the RA pathway. Although this typical range for the nuclear “mechanostat” is a fraction of the lamin-A:B range found across adult tissues, fibroblasts with abundant lamin-A not only lose nearly all of it when reprogrammed to induced pluripotent stem cells (iPSCs) (80) but also are more efficiently reprogrammed (>20-fold) by RA agonists (81) that our results suggest suppress lamin-A. Modifiers beyond the RA pathway could thus couple to the lamin mechanostat and further amplify its range up or down to better match microenvironment stiffness and stress.

Box 1. Polymer physics of the nuclear lamina as a shock absorber

Lamin-A's nonlinear contribution to viscosity in nuclear mechanics relative to lamin-B's contribution to solidlike elasticity can be understood from polymer physics theory. Such theories focus on averages (e.g., mean concentration) while neglecting heterogeneities in molecular states such as polydispersity (e.g., lamin-A splice-forms). For a viscoelastic object such as the model of Fig. 7F, the response time in elongation (92) is

$$\tau = (\text{Viscosity/Elasticity})$$

This is the time required for the energy stored upon rapid stretching of the elastic component to be dissipated by the viscous component. For large polymers, τ is typically seconds to minutes as opposed to nanoseconds for small solutes (92). Moreover, as a function of concentration *c* of polymers ranging from filamentous proteins and DNA to synthetic polymers that are concentrated enough to interact (not dilute), theory and experiment show that Viscosity ~ c^a with scaling exponent $a = 3 \pm 1$ (93, 94). Simple polymer physics therefore predicts that Viscosity ~ [Lamin-A]³. The elasticity of a polymer network such as a lamin-B network, is expected to be proportional to chain density, and so Elasticity ~ [Lamin-B]¹. Therefore

$$\tau_{\text{theory}} \sim [\text{Lamin-A}]^3/[\text{Lamin-B}]^1 = [\text{Lamin-A:B}]^3$$

Experiment gave $\tau \sim [\text{Lamin-A:B}]^{2.5}$ (Fig. 7, G and H). Additional interactions of lamin-A with lamin-B might also affect network elasticity as Elasticity ~ [Lamin-A:B]^{*b*}, and our measurements indicate $b \approx 1$ for $t \rightarrow 0$ (Fig. 7, C to E) and $b \approx 0.5$ on minute time scales. Regardless, the nonlinear scaling result is similar, $\tau_{\text{theory}} \sim c^{3-b} \approx c^{2.5}$. Lamin-A thus contributes to nuclear mechanics primarily as a concentrated polymer that slowly flows when stressed.

Common Structures Under Tension: Supercoiled Helical Assemblies

The α -helical coiled coil and the collagen triple helix are the two known motifs for supercoiled multistranded proteins (82). Both structures tend to assemble into rope-like fibers that are often subjected to tension, but how tension affects structure or turnover is understudied. Collagen-I fibers are degraded more slowly by matrix protease when tension is applied (83), consistent with the well-known atrophy of disuse of connective tissue. The initial findings that lamin-A's filamentous coiled-coil assembly is hyperphosphorylated in cells with low tension on soft matrix is consistent with higher turnover and requires in-depth study but seems to suggest a more general model of biostabilization by tension. Lamin-B1 and -B2 appear less susceptible to this mechanochemical regulation, perhaps because farnesylation of these proteins concentrates them at the nuclear envelope.

Additional structural modulators of supercoil assembly are also likely to contribute to pathophysiological responses. The Ig domain of lamin-A, which affects filament assembly (84) and interacts with a range of nuclear and nuclear-membrane proteins in addition to the SUNs (62) responds conformationally to stress. Phosphorylation at the nearby Ser³⁹⁰, a target of CDK1 (85), was found to differ between wild-type lamin-A and the less stable mutant. Force-driven structural changes in the cytoskeletal protein p130Cas (86) also regulate its phosphorylation by tyrosine kinases, although stress increases phosphorylation of p130Cas and decreases it in lamin-A. Density of lamin-A is ultimately regulated to limit the stress per molecule, thereby adjusting nuclear strength and stiffness consistent with polymer physics and with expectations for a mechanostat. The multifunctionality of a nuclear IF protein such as lamin-A thus results in mechanosensitive feedback on multiple pathways that contribute to differentiation.

Materials and Methods

Whole-Mouse Tissue Lysis

NSG mice (NOD/SCID/IL-2Rγ^{-/-}) were 4 to 8 months in age (*n* = 5 mice). Protocols approved by Penn's Institutional Animal Care and Use Committee (IACUC) were used throughout. Sections of fresh tissue (~50 mm³ heart, brain, liver, lung, kidney, and cartilage from ear) were finely chopped with a razor blade on ice. Additional experiments used flash-frozen tissue (~50 mm³ heart, brain, liver, lung, skeletal muscle, kidney, cartilage from ear, skull, and marrow-free thigh bone), which was finely ground in a pestle and mortar on dry ice. The resulting pastes were suspended in 300-μL ice-cold lysis buffer [1x RIPA buffer (radio immunoprecipitation assay buffer), 1x NuPAGE LDS running buffer (polyacrylamide gel electrophoresis, lithium dodecyl sulfate, from Invitrogen), 0.1% protease inhibitor cocktail (PIC)]. After 30 min incubation at 4°C, samples were subjected to 5 × 20 × 1 s pulses with a probe sonicator (intermediate setting, on ice). A reducing agent was added [β-mercaptoethanol (BME) to 1%] and the samples were heated to 80°C for 10 minutes before ultracentrifugation [1 hour at 90,000 revolutions per minute (rpm) in a TLA120.1 rotor (Beckman) at 4°C]. The blue aqueous layer was then carefully extracted from between layers of fat and any insoluble pelleted material.

Nuclear Enrichment from Mouse Tissue [adapted from (87)]

Sections of flash-frozen tissue (~50 mm³ heart, brain, liver, lung, skeletal muscle, and fat) were thawed on ice, finely chopped with a razor blade and suspended in 10 mL buffer A [10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol (DTT) with 0.1% (PIC, Sigma Aldrich)]. The tissue was then disrupted on ice with a Dounce homogenizer [10 strokes with a

loose-fitting pestle (B grade), 10 strokes with a tight-fitting pestle (A grade)] and persistent detritus was removed by passage through a 40-μm cell filter (Fisher Scientific). Crude nuclear material was then pelleted by centrifugation (10 min at 5000 rpm at 4°C), resuspended in 1 mL buffer S1 (10 mM HEPES, 0.25 M sucrose, 10 mM MgCl₂, and 0.1% PIC) and layered over 1 mL buffer S3 (10 mM HEPES, 1.2 M sucrose, 0.5 mM MgCl₂, and 0.1% PIC). Nuclear material was pelleted by centrifugation [10 min at 5000 rpm in a TLS-55 swinging-bucket rotor (Beckman) at 4°C] and resuspended in 50 μL ice-cold lysis buffer [1x RIPA, 1x NuPAGE LDS running buffer (Invitrogen), 0.1% PIC]. After 30 min incubation at 4°C, samples were subjected to 15 × 1 s pulses with a probe sonicator (low setting, on ice). Before gel electrophoresis, a reducing agent was

added (BME to 1%), heated to 80°C for 10 min, and centrifuged to pellet any insoluble material (10 min at 13,300 rpm).

Mass Spectrometry

Gels from SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (NuPAGE 4 to 12% bis-tris, Invitrogen) were loaded with 12 μL of lysate per lane and run at 10 min × 100 V, followed by 20 min × 160 V. Coomassie-stained polyacrylamide gel sections were washed [50% 0.2 M ammonium bicarbonate (AB) solution, 50% acetonitrile (ACN), 30 min at 37°C], dried by lyophilization, incubated with a reducing agent [20 mM tris(2-carboxyethyl)phosphine (TCEP) in 25 mM AB solution at pH 8.0, 15 min at 37°C], and alkylated [40 mM iodoacetamide (IAM) in 25 mM AB solution at pH 8.0, 30 min at 37°C]. The gel

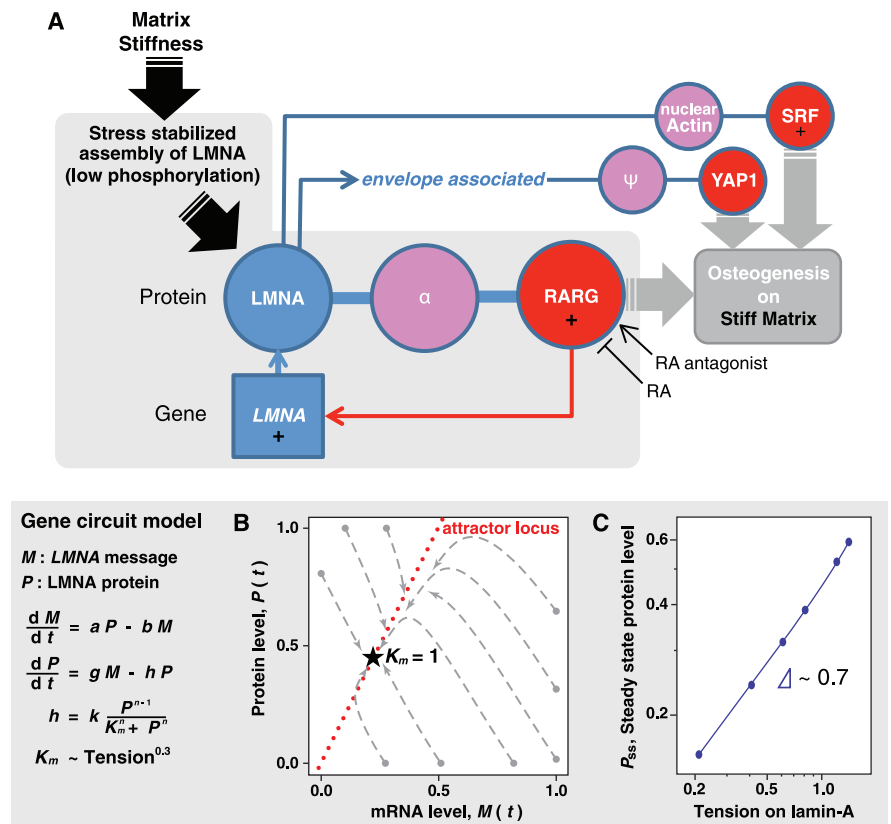


Fig. 8. A feedback-based gene circuit for lamin-A exhibits polymer physics scaling if cell tension suppresses protein turnover. (A) Gene circuit connecting matrix stiffness to osteogenesis; not shown is an overlapping circuit for adipogenesis on soft matrix that includes the positive regulator SREBP1. LMNA protein level is regulated by a stress-sensitive phosphorylation mechanism and feeds back into LMNA transcript through interaction with RARG, possibly through an intermediary, α, and can be perturbed by antagonist (AGN) or agonist (RA). LMNA protein also influences location of YAP1 (through a possible intermediary, Ψ) to drive cell fate (11), and LMNA regulates SRF through interaction with nuclear actin (49). A simple model was generated based on this circuit: Time evolution of LMNA mRNA (*M*) level is dependent on the LMNA protein level (*P*), whereas the protein level itself is regulated by a tension-dependent degradation term, *h*. The model shows that tension-regulated protein turnover can produce steady-state (SS) protein levels that scale with cell tension. (B) Trajectories of lamin-A message and protein as the model converges from a range of initial conditions to a single steady-state solution appropriate to the tension. (C) Setting the kinase/protease binding coefficient, *K_m*, to be proportional to (Tension)^{0.3} allows the model to generate steady-state lamin scaling with tension consistent with experiment (Fig. 1D).

sections were dried by lyophilization before in-gel trypsinization [20 µg/mL sequencing grade modified trypsin in buffer as described in the manufacturer's protocol (Promega), 18 hours at 37°C with gentle shaking]. The resulting solutions of tryptic peptides were acidified by the addition of 50% digest dilution buffer (60 mM AB solution with 3% methanoic acid).

Peptide separations (5 µL injection volume) were performed on 15-cm PicoFrit column (75 µm inner diameter, New Objective) packed with Magic 5 µm C18 reversed-phase resin (Michrom Bioresources) using a nanoflow high-pressure liquid chromatography system (Eksigent Technologies), which was coupled online to a hybrid LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) via a nanoelectrospray ion source. Chromatography was performed with solvent A (Milli-Q water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). Peptides were eluted at 200 nL/min for 3 to 28% B over 42 min, 28 to 50% B over 26 min, 50 to 80% B over 5 min, and 80% B for 4.5 min before returning to 3% B over 0.5 min. To minimize sample carryover, a fast blank gradient was run between each sample. The LTQ-Orbitrap XL was operated in the data-dependent mode to automatically switch between full-scan MS [in terms of mass m and charge z : $m/z = 350$ to 2000 in the Orbitrap analyzer (with resolution of 60,000 at m/z 400)] and the fragmentation of the six most intense ions by collision-induced dissociation in the ion-trap mass analyzer.

Raw mass spectroscopy data was processed using Elucidator (version 3.3, Rosetta Biosoftware). The software was set up to align peaks in data from samples derived from corresponding molecular weight regions of the 1D gels. Peptide and protein annotations were made using SEQUEST (version 28, Thermo Fisher Scientific) with full tryptic digestion and up to two missed cleavage sites. Peptide masses were selected between 800 and 4500 amu with peptide mass tolerance of 1.1 amu and fragment ion mass tolerance of 1.0 amu. Peptides were searched against a database compiled from UniRef100 (November 2010) mouse and human, plus contaminants and a reverse decoy database. A deltaCn of 0.01 and mass error limit of 20 parts per million (ppm) was used, resulting in a false positive rate of ~10%. In these experiments, only proteins detected with three or more peptides were considered. [Therefore, for positive identification of a protein, $P < (0.1)^3$.] The peptide database was modified to search for alkylated cysteine residues (monoisotopic mass change, $\Delta = +57.021$ Da) and oxidized methionine ($\Delta = +15.995$ Da). In proteomic profiling experiments, we also considered the acetylation of lysine ($\Delta = +42.011$ Da), methylation of lysine and arginine ($\Delta = +14.016$ Da), hydroxylation of lysine, proline, aspartate, and asparagine ($\Delta = +15.995$ Da), and phosphorylation of serine, tyrosine, threonine, histidine, and aspartate ($\Delta = +79.966$ Da). In experiments in which cysteine residues were labeled with monobromobimane

(mBBR), the modification was searched for in three possible oxidation states ($\Delta = +133.053$ Da, $\Delta = +150.056$ Da, and $\Delta = +151.064$ Da). Peptides derived from the autolysis of trypsin were considered to be contaminants and were not used in subsequent calculations. When evaluating total ion current, only signals from annotated peptides were summed. The Peptide Ratio Fingerprint (PRF) algorithm was coded for Mathematica (version 8, Wolfram Research) and was used for all MS protein quantitation (88). Synthetic, HPLC-purified versions of key peptides were purchased from GenScript (figs. S4 and S8, E and F) and were used to confirm MS detection and linearity of response.

Determination of Absolute Lamin Ratio (A + C)/(B1 + B2)

When comparing the lamin composition in samples from two conditions—for example, a control and a knockdown—MS analysis allowed the relative (fold) change in lamin level to be established in the lamin-A,C overlap region, lamin-B1, lamin-B2, and an overlap peptide that is common to all lamins, LLEGEER (fig. S4). These ratios were combined to calculate the absolute ratio between A-type and B-type lamin

$$\left(\frac{[\text{Lamin A}]}{[\text{Lamin B}]}\right)_{\text{condition1}} = \frac{f_{\text{LLEGEER}} - f_{\text{Lamin B1, B2}}}{f_{\text{Lamin A}} - f_{\text{LLEGEER}}}$$

where f represents the fold change in protein level in going from condition 1 (e.g., the control) to condition 2 (e.g., the knockdown). Because this measurement was dependent on detection of a single peptide, it was repeated a number of times to obtain a confident measure of the A:B ratio in condition 1. Subsequent derivation of the ratio in condition 2 had no additional dependence on detection of LLEGEER.

$$\left(\frac{[\text{Lamin A}]}{[\text{Lamin B}]}\right)_{\text{condition2}} = \left(\frac{[\text{Lamin A}]}{[\text{Lamin B}]}\right)_{\text{condition1}} \times \frac{f_{\text{Lamin A}}}{f_{\text{Lamin B1, B2}}}$$

The parameter $f_{\text{Lamin B1, B2}}$ represents the overlap between the two B-type lamins. Although we show that lamin-B1 and lamin-B2 were detected in similar quantities and that neither change to a great extent compared with lamin-A,C (Fig. 1F and fig. S4D), we derived this value from median ion current values.

$$R = \frac{\text{Median ion current}_{\text{Lamin B1}}}{\text{Median ion current}_{\text{Lamin B2}}}$$

$$f_{\text{Lamin B1, B2}} = \frac{R * f_{\text{Lamin B1}} + f_{\text{Lamin B2}}}{1 + R}$$

The tissue experiments shown in Fig. 1D were measured relative to a “condition 1” average of brain and heart denoted as <brain, heart>, a reference point chosen in the middle of the elasticity scale for which we had many tissue duplicates ($n = 4$ mice for heart and brain). A

lysate of A549 cells ($n = 3$ culture replicates) was used as a reference point for all cell measurements in Fig. 1D. MS measurements of isolated nuclear material were made in technical triplicates.

Fluid Shear on Isolated Nuclei

Isolated A549 nuclei suspended in nuclei wash buffer were diluted to about 250 nuclei per µL. Nuclei samples were labeled with 150 µM mBBR and immediately loaded into a cone and plate rheometer (Bohlin Gemini) with the stage heated to 37°C. To control for baseline labeling at this temperature an identical sample was placed in a 37°C water bath. Samples were spun in the rheometer for 10 to 40 min with a 10-µm gap. Shear force varied from 0.5 to 5 Pa in different samples. After the run, labeling in both sample and control was quenched with 2 mM glutathione. For each condition, a sample was taken to be imaged. Samples were prepared for MS as described above. To facilitate identification of the labeled lamin Ig domain peptide by MS, recombinantly expressed, mBBR labeled, and trypsinized lamin Ig domain was spiked into control A549 lysate.

Labeling Cysteines with mBBR in Adherent Cells

Low-passage primary human MSCs were seeded on soft (0.3 kPa), intermediate (10 kPa), and stiff (40 kPa) polyacrylamide gels and cultured for 2 days in Dulbecco's minimum essential medium (Invitrogen) supplemented with 10% fetal bovine serum. A549 cells cultured on plastic were transfected with wild-type and R453W-mutant GFP-lamin constructs using Lipofectamine 2000 (LF2k, Invitrogen) per the manufacturer's protocol. Cells were washed with phosphate-buffered saline (PBS) before labeling with freshly prepared 400 µM mBBR in PBS at 37°C. After 10 min, the cells were harvested by trypsinization, suspended in ice-cold media, pelleted, and frozen at -20°C. Samples were enriched before MS analysis by immunoprecipitation of lamin-A or GFP, as described below. In addition, a portion of cells were washed thoroughly after mBBR labeling, fixed with formaldehyde, and immunostained for lamin-A (mouse monoclonal sc-7292, Santa Cruz), α -smooth muscle actin (mouse monoclonal A5228, Sigma Aldrich), and nonmuscle myosin-IIA (rabbit polyclonal M8064, Sigma Aldrich) for imaging at high resolution.

Model of Lamin Transcript and Protein Levels

The rate equations for lamin-A protein (P) and mRNA (M) concentrations, respectively, include synthesis and degradation

$$\frac{dM}{dt} = aP - bM$$

$$\frac{dP}{dt} = gM - hP$$

where a is first-order protein-induced mRNA production rate constant, b is first-order mRNA degradation rate constant, g is first-order mRNA

translational rate constant, h is force-dependent protein degradation, modeled as

$$h = k \frac{P^{n-1}}{K_m^n + P^n}$$

where k is maximal protein degradation rate, n is cooperativity coefficient ≥ 2 typical of dimer-based interactions, and K_m is affinity of kinase/protease for the lamin meshwork, which increases with stress or tension sustained by the meshwork. Like pulling on a coiled rope, the key idea is that tension on this meshwork of lamin-A coiled-coil protein squeezes out free volume and sequesters the enzyme's binding site on lamin-A. At steady-state

$$\frac{dP}{dt} = \frac{dM}{dt} = 0$$

thus yielding nonzero steady-state values for P and M

$$\{P_{ss}, M_{ss}\} = \left\{ \frac{bk}{ga} - \frac{1}{2} \sqrt{\left(\frac{bk}{ga}\right)^2 - 4K_m}, \frac{aP_{ss}}{b} \right\}$$

Based on the steady-state analysis above, a solution only exists if

$$\left(\frac{bk}{ga}\right)^2 - 4K_m > 0$$

Time evolution of P and M was modeled in Mathematica (Wolfram) with example trajectories in Fig. 8B as a phase plot of $P(t)$ versus $M(t)$ converging to $\{P_{ss}, M_{ss}\}$. Although steady-state values depend on the various rate constants, we assumed all to be important and of order ~ 1 as we focus on K_m : At high stresses where lamin-A assembly is favored, K_m increases so that lamin-A phosphorylation/degradation decreases. Plotting P_{ss} against different values for K_m fit a power-law $P_{ss} \sim K_m^2$. If $K_m = (\text{tension})^{0.3}$, then $P_{ss} \sim (\text{tension})^{0.7}$, as found experimentally for lamin-A (Fig. 8C).

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Supplementary Materials

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Materials and Methods
Figs. S1 to S16
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Poverty Impedes Cognitive Function

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The poor often behave in less capable ways, which can further perpetuate poverty. We hypothesize that poverty directly impedes cognitive function and present two studies that test this hypothesis. First, we experimentally induced thoughts about finances and found that this reduces cognitive performance among poor but not in well-off participants. Second, we examined the cognitive function of farmers over the planting cycle. We found that the same farmer shows diminished cognitive performance before harvest, when poor, as compared with after harvest, when rich. This cannot be explained by differences in time available, nutrition, or work effort. Nor can it be explained with stress: Although farmers do show more stress before harvest, that does not account for diminished cognitive performance. Instead, it appears that poverty itself reduces cognitive capacity. We suggest that this is because poverty-related concerns consume mental resources, leaving less for other tasks. These data provide a previously unexamined perspective and help explain a spectrum of behaviors among the poor. We discuss some implications for poverty policy.

A variety of studies point to a correlation between poverty and counterproductive behavior. The poor use less preventive health care (1), fail to adhere to drug regimens (2), are tardier and less likely to keep appointments (3, 4), are less productive workers (5), less attentive parents (6), and worse managers of their finances (7–9). These behaviors are troubling in their own right, but they are particularly troubling because they can further deepen poverty. Some explanations of this correlation focus on the environmental conditions of poverty. Predatory lenders in poor areas, for example, may create high-interest-rate borrowing, and unreliable transportation can cause tardiness and absenteeism. More generally, poverty may leave less room for error so that the “same” mistake can lead to worse outcomes (10, 11). Other explanations focus on the characteristics of the poor themselves. Lower levels of formal education, for example, may create misunderstandings about contract terms, and less parental attention may influence the next generation’s parenting style.

We propose a different kind of explanation, which focuses on the mental processes required by poverty. The poor must manage sporadic income, juggle expenses, and make difficult trade-offs. Even when not actually making a financial decision, these preoccupations can be present and distracting. The human cognitive system has limited capacity (12–15). Preoccupations with pressing budgetary concerns leave fewer cognitive resources available to guide choice and action. Just as an air traffic controller focusing on a po-

tential collision course is prone to neglect other planes in the air, the poor, when attending to monetary concerns, lose their capacity to give other problems their full consideration.

This suggests a causal, not merely correlational, relationship between poverty and mental function. We tested this using two very different but complementary designs (16, 17). The first is a laboratory study: We induced richer and poorer participants to think about everyday financial demands. We hypothesized that for the rich, these run-of-the-mill financial snags are of little consequence. For the poor, however, these demands can trigger persistent and distracting concerns (18, 19). The laboratory study is designed to show that similarly sized financial challenges can have different cognitive impacts on the poor and the rich. But, the study cannot fully capture our hypothesis that in the world, the poor face more challenging demands. In principle, the cognitive impact in situ may be different given that the scale of the problems can vary between the rich and the poor. Perhaps the rich in the world face

larger monetary problems that also cause greater load. Perhaps the poor manage to restructure their lives so that they do not face as many cognitively challenging problems. Put simply, the laboratory study, although illustrating the mechanism, does not show its relevance in natural settings.

Our second study takes a different approach and allows us to assess what happens when income varies naturally. We conducted a field study that used quasi-experimental variation in actual wealth. Indian sugarcane farmers receive income annually at harvest time and find it hard to smooth their consumption (20). As a result, they experience cycles of poverty—poor before harvest and richer after. This allows us to compare cognitive capacity for the same farmer when poor (pre-harvest) versus richer (post-harvest). Because harvest dates are distributed arbitrarily across farmers, we can further control for calendar effects. In this study, we did not experimentally induce financial concerns; we relied on whatever concerns occurred naturally. We were careful to control for other possible changes, such as nutrition and work effort. Additionally, we accounted for the impact of stress. Any effect on cognitive performance then observed would thus illustrate a causal relationship between actual income and cognitive function in situ. As such, the two studies are highly complementary. The laboratory study has a great deal of internal validity and illustrates our proposed mechanism, whereas the field study boosts the external validity of the laboratory study.

We note two observations about these studies. First, they sidestep the discussion on whether poverty is best defined in absolute or relative terms (21). Because our hypothesis is about how monetary concerns tax the cognitive system, we define poverty broadly as the gap between one’s needs and the resources available to fulfill them. Because this is based on subjective needs, it encompasses low-income individuals both in the developing and the developed world as well as those experiencing sharp transitory income shocks, such

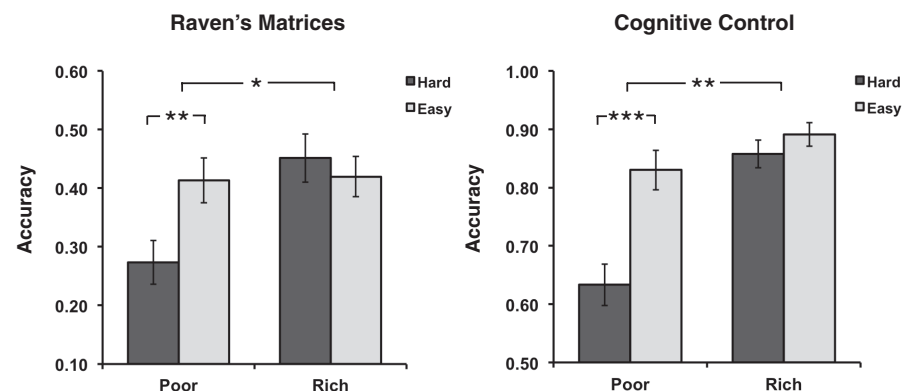


Fig. 1. Accuracy on the Raven's matrices and the cognitive control tasks in the hard and easy conditions, for the poor and the rich participants in experiment 1. (Left) Performance on the Raven's Matrices task. **(Right)** Performance on the cognitive control task. Error bars reflect ± 1 SEM. Top horizontal bars show two-way interaction (poor versus rich $\times$ hard versus easy). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

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as the unemployed. Second, existing theory and data suggest a possibly cumulative long-term impact of poverty on cognition (22, 23): Childhood poverty may hinder brain development and eventually reduce adult cognitive capacity (24). Our hypothesis and tests focus on an immediate impact of poverty on cognition: Budgetary preoccupations can in real time impede cognitive function. Our proposed mechanism does not operate through brain development at early childhood but through an immediate cognitive load caused by financial concerns. Whether this mechanism also contributes to the long-term impacts is an open question.

The Laboratory Studies

The first study consisted of four experiments, with shoppers at a New Jersey mall who participated for pay (details are available in the supplementary materials). This sample encompasses a diverse income range, with the median household income at roughly \$70,000 and a lower bound of roughly \$20,000. This, broadly speaking, provides a cross-section of the United States, with

the poor in our sample roughly corresponding to those in the lower quartile or third of the U.S. income distribution. We computed effective income by dividing household income by the square root of household size (25) and defined “rich” and “poor” through a median split on this variable (26).

In experiment 1, participants ($n = 101$) were presented with four hypothetical scenarios a few minutes apart. Each scenario described a financial problem the participants might experience. For example: “Your car is having some trouble and requires \$X to be fixed. You can pay in full, take a loan, or take a chance and forego the service at the moment... How would you go about making this decision?” These scenarios, by touching on monetary issues, are meant to trigger thoughts of the participant’s own finances. They are intended to bring to the forefront any nascent, easy to activate, financial concerns.

After viewing each scenario, and while thinking about how they might go about solving the problem, participants performed two computer-based tasks used to measure cognitive function:

Raven’s Progressive Matrices and a spatial compatibility task. The Raven’s test involves a sequence of shapes with one shape missing (27). Participants must choose which of several alternatives best fits in the missing space. Raven’s test is a common component in IQ tests and is used to measure “fluid intelligence,” the capacity to think logically and solve problems in novel situations, independent of acquired knowledge (28, 29). The spatial incompatibility task requires participants to respond quickly and often contrary to their initial impulse. Presented with figures on the screen, they must press the same side in response to some stimuli but press the opposite side in response to others. The speed and accuracy of response measures cognitive control (30), the ability to guide thought and action in accordance with internal goals (31). Both are nonverbal tasks, intended to minimize the potential impact of literacy skills. Upon completion of these tasks, participants responded to the original scenario by typing their answers on the computer or speaking to a tape recorder and then moved on to the next scenario (an analysis of participants’ responses to the scenarios is available in table S1). We also collected participants’ income information at the end of the experiment.

Participants were randomly assigned either to a “hard” condition, in which the scenarios involved costs that were relatively high (for example, the car would require \$1500 to fix); or to an “easy” condition, where costs were lower (for example, the car would require \$150 to fix). Because the sums in the easy condition are small, we expected this condition to evoke few of one’s own monetary concerns, for either poor or rich participants. In contrast, the large sums in the hard condition, we hypothesized, would evoke monetary concerns in the poor but not in the rich participants.

Cognitive performance in experiment 1 is plotted in Fig. 1. For the financially “easy” scenarios, designed to generate relatively trivial concerns, the poor and rich performed similarly [Raven’s: $t(50) = 0.13$, $P = 0.90$; cognitive control: $t(50) = 1.55$, $P = 0.13$]. In contrast, in the context of the financially “hard” condition, the poor performed significantly worse than did the rich on both Raven’s [$t(47) = 3.21$, $P < 0.01$] and on cognitive control [$t(47) = 5.22$, $P < 0.001$]. A two-way analysis of variance revealed a robust interaction between income and condition [Raven’s: $F(1,97) = 5.12$, $P = 0.03$; cognitive control: $F(1,97) = 7.86$, $P < 0.01$]. In both tasks, the rich were uninfluenced by condition [Raven’s: $t(48) = 0.56$, $P = .58$; cognitive control: $t(48) = 1.04$, $P = 0.30$], whereas the poor performed significantly worse in the hard condition [Raven’s: $t(49) = 2.63$, $P = 0.01$; cognitive control: $t(49) = 3.98$, $P < 0.001$]. As a result, the poor performed reliably worse than the rich performed overall [Raven’s: $F(1,97) = 5.61$, $P = 0.02$; cognitive control: $F(1,97) = 23.24$, $p < 0.001$]. The magnitudes of the effect here are substantial, with Cohen’s d in this and ensuing replications ranging between 0.88 and 0.94.

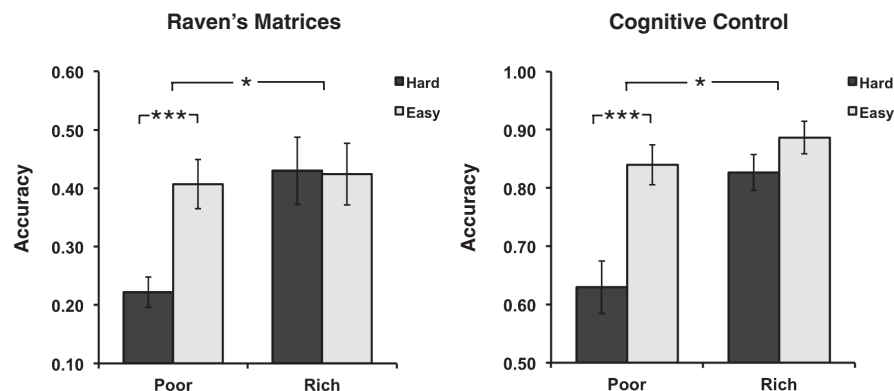


Fig. 2. Accuracy on the Raven’s matrices and the cognitive control tasks in the hard and easy conditions, for the poor and the rich participants, when incentives were provided in experiment 3. (Left) Performance on Raven’s Matrices task. **(Right)** Performance on cognitive control task. Error bars reflect ± 1 SEM. Top horizontal bars show two-way interaction (poor versus rich $\times$ hard versus easy). * $P < 0.05$, *** $P < 0.001$.

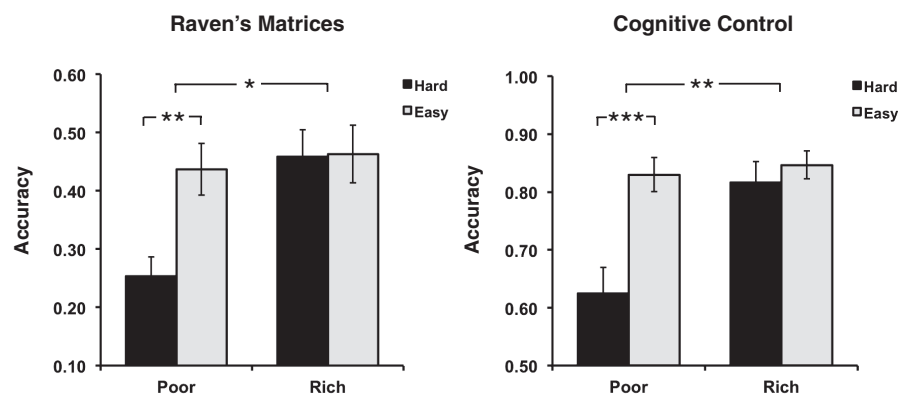


Fig. 3. Accuracy on the Raven’s matrices and the cognitive control tasks in the hard and easy conditions, for the poor and the rich participants in experiment 4. (Left) Performance on Raven’s Matrices task. **(Right)** Performance on cognitive control task. Error bars reflect ± 1 SEM. Top horizontal bars show two-way interaction (poor versus rich $\times$ hard versus easy). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

To rule out the effect of “math anxiety,” experiment 2 used the same set of numbers as in experiment 1 but with nonfinancial scenarios. This recreates a mathematical problem but without evoking financial concerns. There was no interaction between the difficulty of the scenario and participants’ income (further details are available in supplementary materials, experiment 2). Thus, the reduced cognitive performance in the poor participants in experiment 1 was not due to anxiety with large numbers.

Experiment 3 added incentives to experiment 1: In addition to the standard participation fee, participants earned \$0.25 for every correct response on both tasks. Performance in experiment 3 ($n = 100$ participants) is summarized in Fig. 2. As before, the poor performed similarly to the rich in the easy condition [Raven’s: $t(46) = 0.26$, $P = 0.79$; cognitive control: $t(46) = 1.02$, $P = 0.31$] and worse in the hard condition [Raven’s: $t(50) = 3.34$, $P < 0.01$; cognitive control: $t(50) = 3.54$, $P < 0.001$]. The rich performed equally well in the easy and hard conditions [Raven’s: $t(45) = 0.07$, $P = 0.94$; cognitive control: $t(45) = 1.42$, $P = 0.16$], whereas the poor performed significantly worse in the hard condition [Raven’s: $t(51) = 3.75$, $P < 0.001$; cognitive control: $t(51) = 3.67$, $P < 0.001$], yielding a robust interaction between income and scenario [Raven’s: $F(1,96) = 4.34$, $P = 0.04$; cognitive control: $F(1,96) = 4.31$, $P = 0.04$]. Despite the incentives, and the fact that they presumably needed the money more, the poor performed worse overall [Raven’s: $F(1,96) = 6.55$, $P = 0.01$; cognitive control: $F(1,96) = 11.88$, $P < 0.001$] and earned 18% (\$0.71) less than the rich earned.

The hypothetical scenarios are intended to trigger participants’ financial concerns. Yet in experiments 1 to 3, the cognitive tests themselves may have created additional load because they were performed while the participant was contemplating the scenarios. To rule this out, experiment 4 ($n = 96$ participants) replicated experiment 1, except that participants finished responding to each scenario before proceeding to the Raven’s and cognitive control tasks. That is, participants viewed each scenario as in experiment 1, responded to the scenario, and only then completed the Raven’s and cognitive control tasks. Because there were no intervening tasks between scenario presentation and response, we added a few scenario-relevant questions in order to equate the time spent with that of experiment 1. Performance is summarized in Fig. 3.

The results match those in experiments 1 and 3. As before, there was a robust interaction between income and condition [Raven’s: $F(1,92) = 4.04$, $P = 0.04$; cognitive control: $F(1,92) = 6.66$, $P = 0.01$]; the rich and poor performed similarly in the easy condition [Raven’s: $t(48) = 0.41$, $P = 0.69$; cognitive control: $t(48) = 0.43$, $P = 0.67$], and the poor performed significantly worse than the rich performed in the hard condition [Raven’s: $t(44) = 3.55$, $P < 0.001$; cognitive control: $t(44) = 3.34$, $p = .002$]. Condition was insignificant for

the rich [Raven’s: $t(47) = 0.08$, $P = 0.93$; cognitive control: $t(47) = 0.72$, $P = 0.47$], but significant for the poor [Raven’s: $t(45) = 3.26$, $P = 0.002$; cognitive control: $t(59) = 3.94$, $P < 0.001$]. Again, the poor performed worse than the rich performed overall [Raven’s: $F(1,92) = 6.42$, $P = 0.01$; cognitive control: $F(1,92) = 8.74$, $P = 0.004$].

Although remarkably consistent, these findings have limitations. The causal attribution made possible by laboratory studies comes at the expense of some external validity. For example, in experi-

ment 4 the hypothetical scenarios themselves—even after answers were given—may still have weighed on people’s minds. More generally, in all the experiments we explicitly primed monetary concerns. Such explicit priming may not mirror naturally occurring circumstances. It is possible that environments in which one is richer bring to mind other concerns (such as bigger purchases), creating load comparable with that experienced by the poor. It is also possible—though less plausible—that the poor structure their lives to avoid these

Table 1. Changes in financial situation and cognitive capacity around harvest. This table presents changes in farmers’ financial situation (panel A) and their cognitive capacity (panel B) before and after harvest. Each coefficient reported here is the result of an ordinary least-squares regression for the dependent variable in the row heading. For instance, row 1 in column 1 shows that on average, a farmer is 56.6% less likely to have pawned his belongings in the 15-day interval before the post-harvest survey than in the same time interval before the pre-harvest survey. These coefficients also account for any differences that may be attributed to the specific months in which tests were taken. Column 1 reports results for the entire sample; column 2 reports results for farmers who had already completed the harvesting process, but had not yet been paid for the harvest, at the time of the first-round survey. Each cell is the coefficient γ from a separate regression of the type $y_{it} = \alpha_i + \beta_t + \gamma \text{PostHarvest}_{it}$, where the dependent variable varies in each row. Here, i denotes individuals, t denotes time, y denotes various outcome variables, and PostHarvest is a dummy for whether the observation occurs after harvest. The variables α and β reflect a set of individual and time fixed effects, respectively, controlling for all fixed differences between time periods (months) and individuals. Robust standard errors are in square brackets. *Significant at 10%; **significant at 5%; ***significant at 1%. Main independent variable = 1 for the post-harvest period and 0 pre-harvest.

Dependent variable	Full sample: Household + time fixed effects	Subsample: Farmers who completed harvest, but had not received payment
	Column 1	Column 2
Panel A		
Belongings pawned (last 15 days: 0 = no, 1 = yes)	−0.566*** [0.058]	−0.598 [0.058]
Observations	924	630
Mean: 0.41 (0.78 pre-harvest, 0.04 post-harvest)		
Loans outstanding (0 = no, 1 = yes)	−0.885*** [0.033]	−0.899 [0.032]
Observations	922	626
Mean: 0.56 (0.99 pre-harvest, 0.13 post-harvest)		
Number of loans outstanding	−1.979*** [0.105]	−2.033*** [0.106]
Observations	920	626
Mean: 1.22 (2.28 pre-harvest, 0.15 post-harvest)		
Ability to cope with ordinary bills in the past 15 days (1 = low; 3 = high)	0.111*** [0.049]	0.109*** [0.050]
Observations	924	630
Mean: 1.69 (1.62 pre-harvest, 1.76 post-harvest)		
Panel B		
Raven’s accuracy (Min = 0; max = 10)	1.367*** [0.256]	1.321*** [0.274]
Observations	920	624
Mean: 4.9 (4.35 pre-harvest, 5.45 post-harvest)		
Stroop-time taken (In seconds)	−30.582*** [5.923]	−32.319*** [6.208]
Observations	904	618
Mean: 138.94 (146.05 pre, 131.83 post-harvest)		
Stroop-number of errors	−1.818*** [0.566]	−1.937*** [0.588]
Observations	906	620
Mean: 5.55 (5.93 pre, 5.16 post-harvest)		

concerns. To address these issues, we conducted the field study.

The Field Studies

Our second study examined 464 sugarcane farmers living in 54 villages in the sugarcane-growing areas around the districts of Villupuram and Tiruvannamalai in Tamil Nadu, India. These were a random sample of small farmers (with land plots of between 1.5 and 3 acres) who earned at least 60% of their income from sugarcane and were interviewed twice—before and after harvest—over a 4-month period in 2010. There were occasional nonresponses, but all of our pre-post comparisons include only farmers we surveyed twice.

A challenge with pre-post comparisons is calendar effects: Differences between months (such as a festival or the weather) can create a spurious correlation. We overcame this through a particular feature of this context: Farmers' harvest (and planting) dates are staggered over a 3- to 5-month period being set by sugar mills with processing capacity constraints. One farmer may harvest, for example, in June, whereas another harvests in August. The same month then is pre-harvest for some farmers and post-harvest for others. This feature allows us to control for calendar effects.

Our data show that farmers indeed faced greater financial pressures pre- as compared with post-harvest: They pawned items at a higher rate (78 versus 4%, $P < 0.001$, $n = 462$ participants) and were more likely to have loans (99 versus 13%, $P < 0.001$, $n = 461$ participants). On average, farmers had 1.97 more loans before harvest than they did after it. They were also more likely to answer "Yes" to the question, "Did you have trouble coping with ordinary bills in the last fifteen days?" before harvest than after (1.62 and 1.76, respectively, on a 3-point scale, where 1 corresponded to low ability and 3 to high ability to cope; $P < 0.001$, $n = 462$ participants). (Regressions adjusted to take out farmer and month fixed effects are shown in Table 1, panel A.)

We again used Raven's to gauge fluid intelligence. For cognitive control, we could not administer the spatial incompatibility task in the

field. Instead, we used a numeric version of the traditional Stroop task, which is appropriate for participants with low literacy rates. In a typical trial, participants would see "5 5 5" and have to quickly respond "3," which is the number of 5s in the sequence, rather than "5" that comes to mind most naturally. Both response speed and error rates were recorded. Each participant performed 75 trials on the numerical Stroop.

Pre- and post-harvest differences on both tests were pronounced and are illustrated in Fig. 4. On Raven's, the farmers scored an average of 5.45 items correct post-harvest but only 4.35 items correct pre-harvest ($P < 0.001$, $n = 460$ participants). On Stroop, they took an average of 131 s to respond to all items post-harvest, as compared with 146 s pre-harvest ($P < .001$, $n = 452$). In addition, the average number of errors the farmers committed was higher before harvest than after (5.93 versus 5.16 errors; $P < .001$, $n = 453$).

We also report results of regressions that control for farmer and month fixed effects (Table 1, panel B). Each cell in Table 1 is a distinct regression. Table 1, column 1 shows that even after regression adjustment, strong pre-post harvest differences remain for both Raven's and Stroop performance. In addition to these pre-post differences, we found that farmers' perceived intensity of how financially constrained they are—as captured by how they rate their ability to cope with ordinary bills in the preceding 15-day period—correlates negatively with performance on Raven's and time taken on Stroop tests (table S2).

Other factors besides income that vary pre- and post-harvest could drive these effects. One major candidate is physical exertion; preparing the land for harvest might involve increased physical labor. Another candidate is anxiety over crop yield; farmers might be preoccupied not with making ends meet but with how much they will earn. In practice, neither is likely to be true in the case of sugarcane farming. Farmers typically use external labor on their lands, and sugarcane crop size can be readily estimated months before harvest. Still, to address this further we observe that there is a several-week delay between physical harvest and the actual receipt of payment. Finan-

cial burdens are only relieved at the time of payment, but labor and anxiety over crop size are fully resolved at the time of harvest. For 316 farmers in our sample, the "pre-harvest" survey was actually post-physical harvest but pre-payment. We reestimated our equation on this subsample as shown in Table 1, column 2, and found highly similar results, which suggests that neither physical exertion nor anxiety pre-harvest drives our results.

Training effects present another potential confound; post-harvest farmers may do better simply because they are taking the test a second time. To address this, we held back 100 randomly selected farmers at the time of initial sampling. These farmers were surveyed for the first time post-harvest, and their scores were compared with the post-harvest scores of the original sample. If our results were due to learning, we would expect these novice farmers to do worse. Instead, we found that they performed similarly on Raven's accuracy and Stroop reaction time (table S3), suggesting no training effect. There is some evidence for training effects on Stroop error rates (table S3), but the overall pattern cannot be attributed to simple test familiarity. Taken together, the two sets of studies—in the New Jersey mall and the Indian fields—illustrate how challenging financial conditions, which are endemic to poverty, can result in diminished cognitive capacity.

We have argued that the attentional demands created by poverty are a plausible mechanism (29). But there could be other mediating factors. Nutrition is one candidate—in the harvest findings, if not in the mall study; farmers may eat less when poor. In 2009, we ran a pilot study with the same design in the districts of Thanjavur, Thiruvavur, Perambalur, and Pudukottai in Tamil Nadu, in which we surveyed 188 farmers and also asked about food consumption. We found similar effects on Stroop (1.47 errors post-harvest versus 2.12 errors pre-harvest; $P = 0.006$ via t test, $n = 111$ participants). Pre-harvest farmers were not eating less; they spent 2663 rupees a month on food pre-harvest and 2592 rupees post-harvest (roughly \$53 and \$52, respectively, not accounting for purchasing power parity). Additionally, the Stroop results persist even in regressions in which food consumption is included as a control variable.

A potential explanation of these findings is stress. Financial concerns could reasonably induce stress in pre-harvest farmers. Indeed, we examined biological stress. In the 2009 study, we collected two biomarkers of stress: heart rate and blood pressure. Both measures showed that the farmers were more stressed before the harvest; heart rate was higher pre-harvest than post-harvest (78.42 versus 76.38; $P = 0.088$ via t test, $n = 188$ participants), and so were diastolic blood pressure (78.70 versus 74.26, $P < 0.001$ via t test, $n = 188$) and systolic blood pressure (128.64 versus 121.56, $P < 0.001$ via t test, $n = 188$).

However, these differences in stress do not explain our findings. When we reestimated the impact of harvest on Stroop performance, controlling

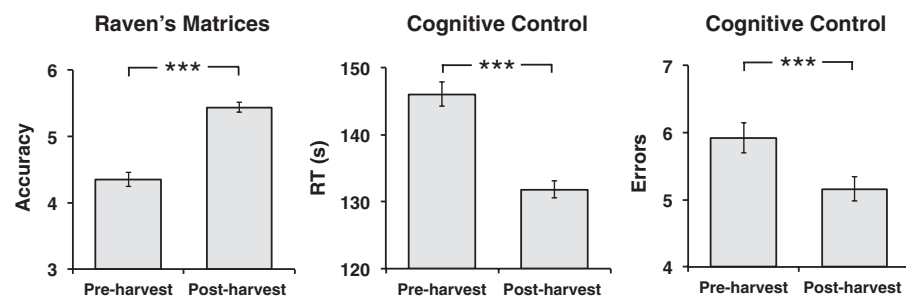


Fig. 4. Accuracy on the Raven's matrices and the cognitive control tasks for pre-harvest and post-harvest farmers in the field study. (Left) Performance on Raven's matrices task. (Middle and Right) Stroop task (measuring cognitive control) response times (RT) and error rates, respectively; error bars reflect ± 1 SEM. Top horizontal bars show test for main effect of pre- versus post-harvest ($***P < 0.001$).

for all three stress measures, the findings remained significant. In fact, the coefficient on post-harvest did not change [for Stroop, we continued to find a coefficient of -1.46 (0.52) on the post-harvest dummy, with a t of -2.80 and $P < 0.006$; $n = 222$ participants]. This suggests that although the pre-harvest farmers did experience stress, stress cannot fully explain the impairment in cognitive function. Our suggested mechanism—that poverty captures attention, triggers intrusive thoughts, and reduces cognitive resources—could itself be described colloquially as “stress”: persistent mental engagement induced by some trigger. The 2009 data, however, suggest that the biological view of stress—as proxied by these biomarkers of stress—is not sufficient to account for our findings. This is consistent with the existing literature on the effects of stress on cognitive function, in which both facilitation and impairment have been found (32). For example, there is evidence that stress can increase working memory capacity (33).

We find attentional capture to be the most compelling explanatory mechanism. It matches findings on the effects of scarcity on borrowing (34) and is consistent with demand and distraction observed in domains of scarcity other than poverty—from insufficient time to limited calorie budgets (35). But surely, other mechanisms might be operating. For example, poverty might influence cognitive load by changing people's affective state (36, 37). We hope future work will test other mechanisms for explaining these findings.

New Perspectives on Policy

The data reported here suggest a different perspective on poverty: Being poor means coping not just with a shortfall of money, but also with a concurrent shortfall of cognitive resources. The poor, in this view, are less capable not because of inherent traits, but because the very context of poverty imposes load and impedes cognitive capacity. The findings, in other words, are not about poor people, but about any people who find themselves poor.

How large are these effects? Sleep researchers have examined the cognitive impact (on Raven's) of losing a full night of sleep through experimental manipulations (38). In standard deviation terms, the laboratory study findings are of the same size, and the field findings are three quarters that size. Put simply, evoking financial concerns has a cognitive impact comparable with losing a full night of sleep. In addition, similar effect sizes have been observed in the performance on Raven's matrices of chronic alcoholics versus normal adults (39) and of 60- versus 45-year-olds (40). By way of calibration, according to a common approximation used by intelligence researchers, with a mean of 100 and a standard deviation of 15 the effects we observed correspond to ~ 13 IQ points. These sizable magnitudes suggest the cognitive impact of poverty could have large real consequences.

This perspective has important policy implications. First, policy-makers should beware of imposing cognitive taxes on the poor just as they

avoid monetary taxes on the poor. Filling out long forms, preparing for a lengthy interview, deciphering new rules, or responding to complex incentives all consume cognitive resources. Policy-makers rarely recognize these cognitive taxes; yet, our results suggest that they should focus on reducing them (41). Simple interventions (41) such as smart defaults (42), help filling forms out (43), planning prompts (44), or even reminders (45) may be particularly helpful to the poor. Policy-makers should further recognize and respond to natural variation in the same person's cognitive capacity. Many programs that impose cognitive demand on farmers, for example, from HIV education to agricultural extension services (which provide farmers with information about new seeds, pesticides, and agricultural practices) should be carefully timed. At the very least, as our results suggest, they should be synchronized with the harvest cycle, with greater cognitive capacity available post-harvest. One recent study illustrated this with fertilizer. Farmers made higher-return investments when the decision was made right after harvest as compared with later in the season (46). The data suggest a rarely considered benefit to policies that reduce economic volatility: They are not merely contributing to economic stability—they are actually enabling greater cognitive resources.

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Supplementary Materials

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Dissecting X-ray–Emitting Gas Around the Center of Our Galaxy

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Most supermassive black holes (SMBHs) are accreting at very low levels and are difficult to distinguish from the galaxy centers where they reside. Our own Galaxy’s SMBH provides an instructive exception, and we present a close-up view of its quiescent x-ray emission based on 3 megaseconds of Chandra observations. Although the x-ray emission is elongated and aligns well with a surrounding disk of massive stars, we can rule out a concentration of low-mass coronally active stars as the origin of the emission on the basis of the lack of predicted iron (Fe) $K\alpha$ emission. The extremely weak hydrogen (H)–like Fe $K\alpha$ line further suggests the presence of an outflow from the accretion flow onto the SMBH. These results provide important constraints for models of the prevalent radiatively inefficient accretion state.

The nucleus of our Galaxy offers a multitude of opportunities for observing the interplay between a supermassive black hole (SMBH) and its immediate surroundings. The SMBH, named Sgr A* (Sagittarius A*), has a mass of 4.1×10^6 solar masses ($M_\odot$) (1, 2), and at its distance of 8 kpc (1), an arc sec (1") subtends 1.2×10^{17} cm, or $1.0 \times 10^5 r_s$, where $r_s = 1.2 \times 10^{12}$ cm is the Schwarzschild radius. The x-ray emission of Sgr A* typically has an unabsorbed 2- to 10-keV luminosity (L_x) of a few times 10^{33} erg s⁻¹ (3), or a factor of $\sim 10^{11}$ lower than the canonical maximum allowed (Eddington) luminosity of the SMBH, representing a common “inactive” state of galactic nuclei in the local universe. Because of its proximity, Sgr A* allows us to study this extremely low- L_x state in unparalleled detail [e.g., (4, 5)].

It is believed that Sgr A* feeds off the winds from surrounding massive stars (6–8). At the so-

called Bondi capture radius $r_B \sim 4''(T_d/10^7 \text{ K})^{-1}$ (9), the gravitational pull of the SMBH overcomes the expanding motion of the medium with effective temperature T_d . The corresponding Bondi capture rate is estimated to be $\dot{M}_B \sim 1 \times 10^{-5} M_\odot \text{ year}^{-1}$ [e.g., (3)]. If Sgr A* indeed accretes at this rate and if the 10% x-ray emission efficiency of a “normal” active galactic nucleus applies, one would predict a luminosity of $L_x \sim 10^{41}$ erg s⁻¹. That the observed L_x is nearly a factor of $\sim 10^8$ smaller has led to a renaissance of radiatively inefficient accretion flow (RIAF) models (10, 11), including self-similar solutions (12–17) and numerous hydrodynamic or magneto-hydrodynamic

simulations of various complexities and dynamic ranges [e.g., (18–23)]. Many of the recent model developments were stimulated by submillimeter polarization and Faraday rotation measurements, providing stringent constraints on the accretion rate in the innermost region ($r \lesssim 10^2 r_s$) of Sgr A* [e.g., (24)]. But controversies remain as to which model (if any) may apply [e.g., (19, 21)].

High-angular resolution observations of Sgr A*—such as those provided by the Chandra X-ray Observatory—can in principle probe the accretion phenomenon not only in its innermost regions, but also the outer boundary conditions at flow onset. However, there are substantial uncertainties regarding the interpretation of the quiescent emission of Sgr A*, which previous Chandra observations showed to be extended with an intrinsic size of about 1.4" (3). How that emission is distributed within this region, and how much is due to a bound accretion flow versus other components, remains unclear. Furthermore, it has been proposed (3, 25) that a substantial or even dominant fraction of the emission could arise from a centrally peaked population of coronally active, low-mass main-sequence stars around Sgr A*, which is allowed by current near-infrared observations. This scenario predicts a 6.4-keV emission line with the equivalent width (EW) in the range of 50 to 100 eV, due to fluorescence of photospheric weakly ionized iron, irradiated by coronal flare x-rays. It is thus essential to test this hypothesis before we can assign the x-ray emission to the accretion flow onto the SMBH.

We use data taken during the Sgr A* X-ray Visionary Program [XVP; (26)]. The Advanced

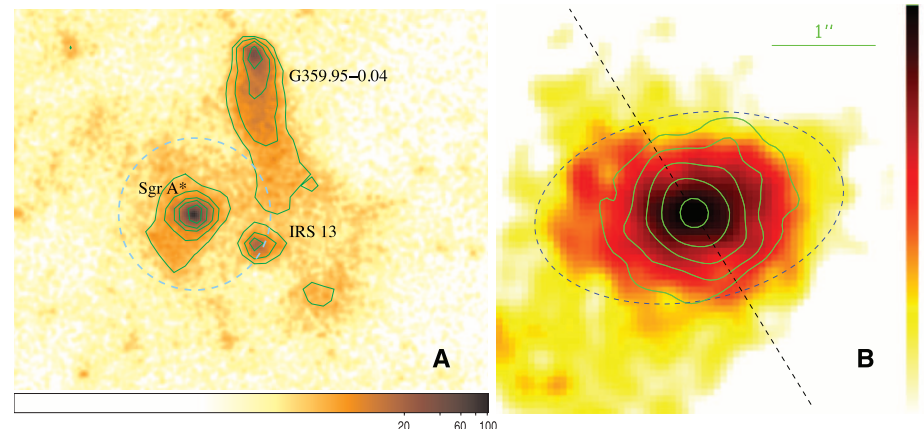


Fig. 1. X-ray images of Sgr A* in quiescence. **(A)** An image constructed with the XVP 0th-order ACIS-S/HETG data in the 1- to 9-keV band. The contours are at 1.3, 2.2, 3.7, 6.3, and 11×10^{-4} counts s⁻¹ arc sec⁻². North is up and east is to the left. The dashed circle around Sgr A* marks its Bondi capture radius (assumed to be 4"). **(B)** A magnified image of Sgr A*. The emission is decomposed into extended (color image) and pointlike (contour) components. The latter component is modeled with the net flare emission (26) and is illustrated as the intensity contours at 0.3, 0.6, 1.2, 2.4, and 5 counts per pixel. The straight dashed line marks the orientation of the Galactic plane, whereas the dashed ellipse of a 1.5" semimajor axis illustrates the elongation of the primary massive stellar disk, which has an inclination of $i \sim 127^\circ$, a line-of-nodes position angle of 100° (east from north), and a radial density distribution $\propto r^{-2}$ with a sharp inner cutoff at $r \approx 1'$ (27).

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CCD (charge-coupled device) Imaging Spectrometer-Spectroscopy (ACIS-S), combined with the High-Energy Transmission Grating (HETG), was placed at the aim point of the telescope. The on-axis spatial resolution of Chandra is $\sim 0.4''$ (full width at half-maximum), whereas the spectral resolution of the 0th-order ACIS-S/HETG is about a factor of ~ 2 better than that of the previous ACIS-I observations and thus enables critical spectroscopic diagnostics of the x-ray emission.

These observations reveal x-ray emission from Sgr A* that appears substantially more extended than a pointlike source [Fig. 1A; (26)]. After subtracting a pointlike contribution, which accounts for 20% of the total quiescent emission (26), an east-west-elongated x-ray enhancement emerges around Sgr A* on $\sim 1''$ to $1.5''$ scales (Fig. 1B). This relatively symmetric enhancement morphologically resembles the so-called clockwise stellar disk, which is the most notable kinematically organized structure known for the massive stars around Sgr A* (27, 28). Irregular low-surface-brightness features (e.g., spurs toward the northeast and southeast; Fig. 1B) appear on scales greater than the enhancement, but still roughly within the Bondi radius. These features are softer (more prominent in the 1- to 4-keV band than in the 4- to 9-keV band) than the smoothly distributed background.

The quiescent x-ray emission is also spectrally distinct from the pointlike flare emission (26), which is considerably harder. The accumulated flare spectrum can be well characterized by a power law with photon index 2.6(2.2, 3.0), and foreground absorption with equivalent hydrogen column density $N_H = 16.6(14.1, 19.4) \times 10^{22} \text{ cm}^{-2}$ [see (29) for the definition of the uncertainties], consistent with the previous analysis of individual bright flares (30, 31). Because of the simplicity of the flare spectrum, this N_H measurement can be considered a reliable estimate of the foreground x-ray-absorbing column density along the sightline toward Sgr A* and hence a useful constraint in modeling the more complex spectrum of the quiescent x-ray emission.

By contrast, the quiescent spectrum shows prominent emission lines (Fig. 2). In addition to the previously known Fe K α emission at ~ 6.7 keV,

K α lines of several other species (He- and H-like Ar, He-like S and Ca), as well as He-like Fe K β , are also apparent. We find that a single-temperature (1-T) plasma with metal abundance set equal to zero describes the overall (continuum) shape of the observed spectrum well, and individual Gaussians can be used to characterize the centroid, flux, and EW of six most noticeable emission lines [Fig. 2A; (26)]. There is little sign of the diagnostic K α emission lines from fluorescent neutral or weakly ionized Fe at ~ 6.4 keV or H-like Fe at 6.97 keV.

We can reject the stellar coronal hypothesis, on the basis of our temporal, spatial, and spectral results. First, our spectrum does not confirm the presence of the 6.4-keV line, previously suggested by (25). Measuring the 6.4-keV line, which is adjacent to the strong highly ionized Fe K α line, is difficult when the spectral resolution is poor, as was the case for the previous ACIS-I spectrum, because the measurement then depends sensitively on the assumed thermal plasma model. Our measured upper limit to the EW of the 6.4-keV line emission, 22 eV (26), is more than a factor of 2 below the range predicted before (25). Second, the quiescent emission shows no appreciable variation on time scales of hours or days, as expected from the sporadic giant coronal flares of individual stars. Third, if the bulk of the quiescent emission is due to stellar coronal activity, then it should also account for some of the detected x-ray flares, especially the relatively long and weak ones (25). However, no sign of any line emission, even the strong Fe K α line, is found in any flare spectra [individual or accumulated; (26, 32)]. Fourth, the spatial distribution of the weak flares is as pointlike as the strong ones, in contrast to the extended quiescent emission (26). Therefore, we conclude that neither flare nor quiescent emission originates from stellar coronal activity, although the latter could still give a small ($\lesssim 25\%$) contribution to the quiescent emission.

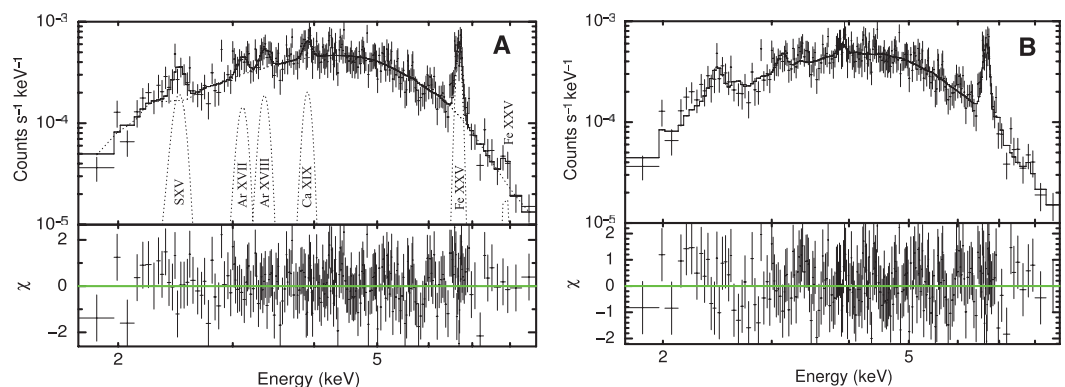
As described above, the diffuse x-ray emission on the scale of $\lesssim 1.5''$ (or $1.5 \times 10^5 r_s$) morphologically resembles a gaseous disk with inclination angle and line-of-nodes similar to those of the stellar disk around Sgr A*. This scale, a factor of $\gtrsim 2$ smaller than r_B , corresponds to the sound-crossing distance over $\sim 10^2 (T_q/10^7 \text{ K})^{-1/2}$ years

(where T_a is the ambient gas temperature), which is about the time since the last major burst of Sgr A*, as inferred from x-ray light echoes [e.g., (33, 34)]. The burst, making Sgr A* a factor of $\sim 10^6$ brighter than its present state over a period of several 10^2 years, could have substantially altered the surroundings, as well as the accretion flow itself. A stable accretion flow would then need to be reestablished gradually, roughly at the sound-crossing speed. This flow would be expected to carry the net angular momentum of the captured wind material with an orientation similar to that of the stars, which could explain the morphological similarities. However, the recent hydrodynamic simulations of the stellar wind accretion (8) suggest that a Keplerian motion-dominated flow should occur on much smaller scales ($r \lesssim 0.1''$). If true, then the plasma on larger scales must be supported mainly by large gradients of thermal, magnetic, cosmic-ray, and/or turbulence pressures, and/or the motion must be strongly affected by the outward transport of angular momentum from the accretion flow, as indicated in various simulations [e.g., (19)].

One can further use the relative strengths of individual lines as powerful diagnostics of the accretion flow (35, 36). X-ray emission lines trace the emission measure distribution as a function of plasma temperature. The ionic fraction of He-like S (S XV), for example, peaks at $k_B T \sim 1.4$ keV (where k_B is Boltzmann's constant and T is temperature), while the He-like Fe dominates in the temperature range of ~ 1.5 to 7 keV and the H-like Fe peaks sharply at ~ 9 keV. At temperatures $\gtrsim 12$ keV, Fe becomes nearly fully ionized. Thus, if the radial dependence of the temperature can be modeled, the density or mass distribution as a function of radius can be inferred.

The extremely weak H-like Fe K α line in the Sgr A* spectrum immediately suggests a RIAF with a very low mass fraction of the plasma at $kT \gtrsim 9$ keV, or a strong outflow at radii $r \gtrsim 10^4 r_s$ (26). Radially resolved analysis further shows that the x-ray spectrum becomes increasingly hard with decreasing radius and that the line centroid and EW of the Fe K α line also changes with the radius. These characteristics can naturally be explained by the presence of plasma at a relatively low temperature in the region, consistent with the

Fig. 2. The x-ray spectrum of Sgr A* in quiescence [extracted from the inner circle of $1.5''$ radius and local-background-subtracted; (26)] and model fits: (A) Zero-metallicity 1-T thermal bremsstrahlung continuum plus various Gaussian lines (table S1); (B) RIAF model with the best-fit $\gamma = 2s/\theta = 1.9$, where s and θ are the indices parametrizing the density and temperature profiles, respectively (26).



onset of the RIAF from captured stellar wind material at $r \sim 10^5 r_s$.

As detailed in (26), we can quantitatively check the consistency of the x-ray spectrum of the quiescent Sgr A* with various RIAF solutions. In particular, we can reject (with a null hypothesis probability of 10^{-6}) a no-outflow solution (i.e., a constant mass accretion rate along the accretion flow leading to a radial plasma density profile $n \propto r^{-3/2+s}$, in which $s = 0$). This solution substantially overpredicts the H-like Fe K α line, and other lines are not fully accounted for. The predicted shape of the model spectrum is also far too flat, resulting in a substantially reduced N_H [$= 8.0 (7.6, 8.3) \times 10^{22} \text{ cm}^{-2}$], inconsistent with other estimates. Therefore, the no-outflow solution can be firmly rejected, both statistically and physically.

We find that a RIAF model with a flat radial density profile (i.e., $s \sim 1$) provides an excellent fit to both the continuum and line data (Fig. 2B) and gives estimates of both the plasma metal abundance and the foreground absorption column consistent with other independent measurements (26). With this fit, we can infer the radial emission structure of the accretion flow. Although the line emission is dominated by the outer, cooler region of the RIAF ($r \gtrsim 10^4 r_s$), the innermost hot component contributes primarily to the continuum emission, mostly via bremsstrahlung processes. We find that this latter contribution accounts for only $\lesssim 20\%$ of the observed quiescent x-ray luminosity (consistent with the spatially decomposed fraction), in stark contrast to the $s = 0$ solution, whose x-ray emission is completely dominated by the innermost regions ($r \lesssim 10^2 r_s$).

Further insight into the physical processes involved in the RIAF can be obtained from comparing our x-ray measurements, most sensitive to the outer radial density profile, with existing constraints on the accretion properties in the innermost region. For example, submillimeter Faraday rotation measurements (24) set a lower limit to the accretion rate of $2 \times 10^{-9} M_\odot \text{ year}^{-1}$ into the innermost region. Assuming that a large fraction of the Bondi accretion rate, $\dot{M}_B \sim 1 \times 10^{-5} M_\odot \text{ year}^{-1}$, initially ends up in the RIAF at a radius $\sim 10^5 r_s$, then an $s \sim 1$ density profile can exist down to radius $r_i \sim 10^2 r_s$. This $s \sim 1$ density profile over a broad radial range is consistent with various RIAF solutions [e.g., (14, 17)] and numerical simulations [e.g., (18)]. The Faraday rotation measurements also yield an upper limit to the accretion rate of $2 \times 10^{-7} M_\odot \text{ year}^{-1}$, assuming that the magnetic field is near equipartition, ordered, and largely radial in the RIAF (24). With this upper limit, assuming that $r_i \sim 10^2 r_s$, we can infer $s > 0.6$ and hence $\theta \gtrsim 0.6$ for the radial temperature profile $T \propto r^{-\theta}$ of the RIAF. This constraint on θ places a fundamental limit on thermal conduction-induced heat outflow (37).

The flat density profile of the flow (i.e., $s \sim 1$) suggests the presence of an outflow that nearly balances the inflow (26). As a result, $\lesssim 1\%$ of the matter initially captured by the SMBH reaches the innermost region around Sgr A*, limiting the

accretion power to $\lesssim 10^{39} \text{ erg s}^{-1}$. Much of this power is probably used to drive the outflow, which could affect the environment of the nuclear region and even beyond [e.g., (38)]. This combination of the low energy generation and high consumption rates of Sgr A* naturally explains its low bolometric luminosity (a few times $10^{36} \text{ erg s}^{-1}$) and its lack of powerful jets. Observations of nearby elliptical galaxies with jet-inflated cavities in diffuse x-ray-emitting gas reveal a relation between the jet and Bondi accretion powers (in units of $10^{43} \text{ erg s}^{-1}$), $P_{\text{jet}} \approx 0.007 P_{\text{Bondi}}^{1.3}$, where P_{Bondi} is assumed to have a 10% efficiency of the Bondi mass accretion rate (39). We speculate that the nonlinearity of this relation is due to increasing outflow rates with decreasing Bondi power, leading to a reduced energy-generation rate in the innermost region around a SMBH, where jets are launched. Extrapolating the relation down to the case for Sgr A*, we may expect a jet-to-Bondi power ratio of only $\sim 0.2\%$, consistent with the above inferred fractional mass loss due to the outflow.

The progress in understanding the accretion flow characteristics and orientation for our closest SMBH will lead to key constraints for the modeling of such phenomena in general. For Sgr A* specifically, these results will influence models of the “shadow” of the SMBH [e.g., (40, 41)], which can be measured by very-long-baseline interferometry at (sub)millimeter wavelengths (42), and the radiation from hydrodynamic interactions of orbiting stars [e.g., S2; (43)] and the G2 object with ambient media at distances down to $\sim 10^3 r_s$ from the SMBH [e.g., (44)].

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Supplementary Materials

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Stretchable, Transparent, Ionic Conductors

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Existing stretchable, transparent conductors are mostly electronic conductors. They limit the performance of interconnects, sensors, and actuators as components of stretchable electronics and soft machines. We describe a class of devices enabled by ionic conductors that are highly stretchable, fully transparent to light of all colors, and capable of operation at frequencies beyond 10 kilohertz and voltages above 10 kilovolts. We demonstrate a transparent actuator that can generate large strains and a transparent loudspeaker that produces sound over the entire audible range. The electromechanical transduction is achieved without electrochemical reaction. The ionic conductors have higher resistivity than many electronic conductors; however, when large stretchability and high transmittance are required, the ionic conductors have lower sheet resistance than all existing electronic conductors.

The emergence of the field of stretchable electronics, along with its biomedical applications, has highlighted a challenge: Electronic devices are usually made of hard materials, whereas tissues and cells are soft. Stretchable conductors are needed to enable electronics to meet skin, heart, and brain (1, 2). Stretchable conductors are also needed in applications, such as electromechanical transduction (3) and solar energy conversion (4). Existing stretchable conductors are mostly electronic conductors, including carbon grease (5), microcracked gold films (6), serpentine-shaped metallic wires (1), carbon nanotubes (7, 8), graphene sheets (9, 10), and silver nanowires (11–13). Stretchable devices have also been made by using corona discharge (14), liquid metals (15), and saline solutions (16); these conductors, however, are not solid, and their uses are limited. Attributes other than conductivity and stretchability are also important in specific applications. Conductors may need to operate at high frequencies and high voltages (5), remain conductive while undergoing areal expansions of 1000% or more (17), be biocompatible (3), and be transparent (7–13).

Whereas electronic conductors struggle to meet these demands, ionic conductors meet most of them readily. Many ionic conductors, such as hydrogels (18) and gels swollen with ionic liquids (19), take a solid form and are stretchable and transparent. Many hydrogels are biocompatible and conformal to tissues and cells down to the molecular scale (20). We demonstrate that ionic conductors can even be used in devices requiring

voltages and frequencies much higher than those commonly associated with devices using ionic conductors.

One basic design places two electrodes (electronic conductors), an electrolyte (ionic conductor), and a dielectric (insulator) in series (Fig. 1A). The electrode/electrolyte interface forms an electrical double layer (Fig. 1B). For some combinations of the electrode and electrolyte, if the voltage across the interface is within a certain range (~1 V), the interface is ideally polarizable—that is, electrons and ions do not cross the interface, no electrochemical reaction occurs, and the electrical double layer behaves like a capacitor (Fig. 1C). A circuit with electronic and ionic conductors in series cannot carry sustained electrical current in the absence of electrochemical reaction.

The electrical double layer and the dielectric are two capacitors in series. They capacitively couple the electrical signals carried by the two electrodes and transport power with alternating current.

The two capacitors have vastly different capacitances. For the electrical double layer, charges in the electrode and in the electrolyte are separated over nanometers; this small separation leads to a large capacitance, on the order of $c_{EDL} \sim 10^{-1} \text{ F/m}^2$ (19). For the dielectric, charges on its two faces are separated over the thickness of the dielectric (millimeters in our experiments); this large separation leads to a small capacitance, on the order of $c_D \sim 10^{-8} \text{ F/m}^2$. At equilibrium, the voltage V applied between the two electrodes is carried entirely by the electrical double layer and the dielectric, $V = V_{EDL} + V_D$ (Fig. 1A). In response to the applied voltage, the two capacitors add the same amount of charge, $c_D A_D V_D = c_{EDL} A_{EDL} V_{EDL}$, where A_{EDL} is the area of the electrical double layer and A_D is the area of the dielectric. In our experiments, $A_{EDL}/A_D \sim 0.01$; the large ratio $c_{EDL}/c_D \sim 10^7$ ensures that the voltage across the electrical double layer V_{EDL} is well below 1 V when the voltage across the dielectric is on the order of $V_D \sim 10 \text{ kV}$. A small V_{EDL} prevents electrochemical reaction, whereas a large V_D enables electromechanical transduction.

We built a transparent, high-speed, large-strain actuator. A membrane of a dielectric elastomer was sandwiched between two membranes of an electrolytic elastomer (Fig. 2A and fig. S1). The dielectric and the electrolyte are stretchable and transparent, but the electrodes need not be. The electrode/electrolyte interfaces can be much smaller than the area of the dielectric, allowing us to place

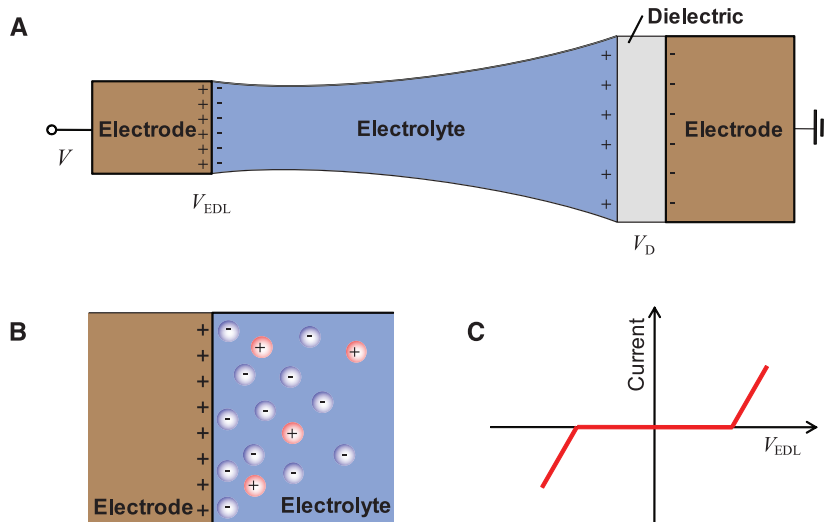


Fig. 1. A basic design of stretchable ionics. (A) An electrolyte and a dielectric are in series. When they are elastomeric, the device is solid and stretchable. When a voltage V is applied between the two electrodes, the voltage across the electrode/electrolyte interface is much smaller than the voltage across the dielectric, $V_{EDL} \ll V_D$. (B) The electrode/electrolyte interface forms an electrical double layer. (C) When the voltage across the interface is within a certain range, electrons and ions do not cross the interface, no electrochemical reaction occurs, and the electrical double layer behaves like a capacitor.

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the electrodes outside the active region. Consequently, the active region of the actuator consisted of only electrolytic and dielectric elastomers and was highly stretchable and transparent. When a voltage was applied between the electrodes, ions of different charge polarities collected on the two faces of the dielectric; the oppositely charged interfaces attracted each other and caused the sandwich to reduce its thickness and enlarge its area (Fig. 2B).

We demonstrated this design by using 1-mm-thick VHB 4910 tape (3M) as the dielectric and 100- μ m-thick polyacrylamide hydrogel containing NaCl as the electrolyte. To compare the performance of the ionic conductor to an electronic conductor, we used the hydrogel to replace the carbon grease in the actuator used in (5). We stacked three layers of VHB together, stretched them radially to three times their initial radius,

and fixed them to a circular acrylic frame (fig. S1). We then sandwiched the dielectric stack between two layers of heart-shaped hydrogels, which are linked, through thin hydrogel lines, to copper electrodes placed on the frame. When a voltage is applied and removed, the heart expands and contracts (movie S1). The beating heart is transparent to all colors (Fig. 2, C and D).

We also fabricated an actuator with the dielectric sandwiched between layers of the hydrogels of circular shape and compared the experimental data with theory (fig. S2). An area strain of up to 167% was achieved (Fig. 2, E and F). This voltage-induced strain was limited by electromechanical instability (fig. S3). Furthermore, the soft hydrogels did not appreciably constrain the dielectric. The area strain of our actuator reduced as the frequency of applied voltage increased and became vanishingly small at a frequency on

the order of 10^3 Hz (Fig. 2F). These characteristics of the actuator using the hydrogel are comparable to those of an actuator using carbon grease (fig. S4), but the carbon grease is an opaque electronic conductor.

The frequency of actuation is not limited by electrical resistance but by mechanical inertia. The time to charge the device, the RC delay, scales as $\tau_{RC} \sim c_D A_D R$, where R is the sheet resistance of the ionic conductor. For representative values $c_D = 10^{-8}$ F/m², $A_D = 10^{-2}$ m², and $R = 10^2$ ohm/square, we estimate that $\tau_{RC} \sim 10^{-8}$ s, corresponding to a frequency much higher than the observed limiting frequency of actuation. The fundamental resonance sets a time $\tau_{inertia} \sim \sqrt{A_D \rho / Y}$, where ρ is the mass density and Y the elastic modulus. For representative values $\rho = 10^3$ kg/m³ and $Y = 10^5$ N/m², we estimate that $\tau_{inertia} \sim 10^{-3}$ s; this value is consistent with the observed limiting frequency of actuation.

To demonstrate that the ionic conductors enable electromechanical transduction much beyond the fundamental resonance, we built a transparent loudspeaker that produces sound across the entire audible range, from 20 Hz to 20 kHz. The loudspeaker was placed in front of a laptop, which played music videos (Fig. 3, A and B, and fig. S5). The screen of the laptop was visible through the loudspeaker. The sound tracks of the videos were fed to the loudspeaker as analog voltage signals, from the audio output of the laptop, through a high-voltage amplifier. A video of the transparent loudspeaker and the sound produced were recorded with a Web camera (webcam) at a distance of 15 cm (movie S2).

We studied the fidelity of the sound reproduction by feeding the loudspeaker with a 20-s test signal of constant amplitude and a linear sine sweep from 20 Hz to 20 kHz (Fig. 3, C and D). The sound generated by the loudspeaker was recorded by the webcam (movie S3). In the first few seconds, the amplitude of the recorded sound was large (Fig. 3E). This interval reflects the resonance of the frame of the loudspeaker, which was not optimized to suppress vibration. The amplitude varied only slightly over the remainder of the recording, where variations in amplitude might be overshadowed by background noise from the high-voltage amplifier. The spectrogram of the recorded sound displayed the successful reproduction of the main signal of the original test sound throughout the audible frequency range (Fig. 3F). In the lower part of the tested frequency range, vibrations of the frame and the membrane were visible (movie S4).

Loudspeakers have been made by using electronic conductors, such as graphene sheets (21), carbon nanotubes (22), carbon grease (23), and conducting polymers (24). Some loudspeakers use piezoelectric polymers (21, 22), and others use dielectric elastomers (23, 24). Pairing the ionic conductors with transparent piezoelectric polymers will take advantage of the high transparency of the ionic conductors; piezoelectric polymers can be operated with lower electric

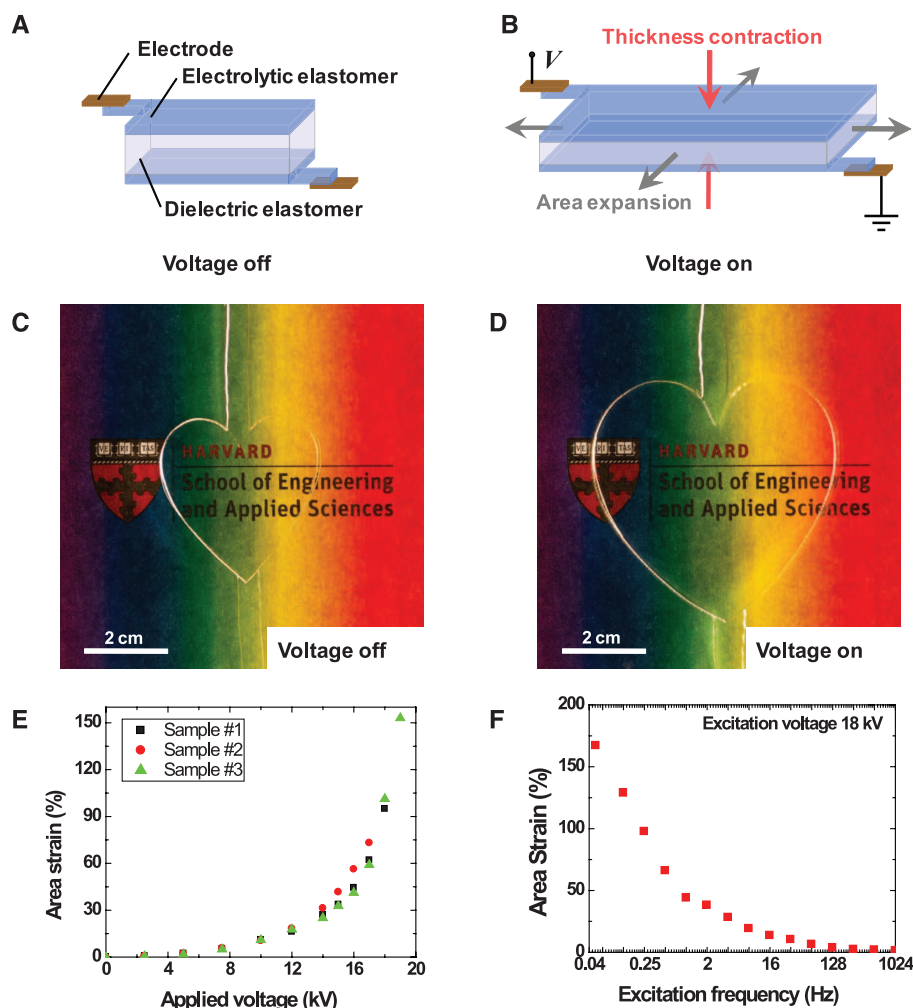


Fig. 2. Transparent actuator capable of fast voltage-induced deformation. (A) A dielectric elastomer is sandwiched between two layers of an electrolytic elastomer. Both the dielectric and the electrolyte are transparent and stretchable, and the device is transparent if the electrodes are placed outside the active area of the device. (B) Subject to voltage, the two layers of the electrolyte spread ions of opposite signs on the two sides of the dielectric, causing the sandwich to reduce thickness and expand area. (C and D) The actuator is transparent to all colors. The area strain is measured as a function of voltage (E) and as a function of the frequency (F) by using an actuator with electrolytes of circular shape.

fields compared with dielectric elastomers and have a linear relation between electric field and strain (21, 22).

The ionic conductors have higher resistivity than many electronic conductors; however, when high stretchability and transmittance are required, the ionic conductors have lower sheet resistance than existing electronic conductors, such as silver nanowires (AgNWs), single-wall carbon nanotubes (SWNTs), graphene, and indium tin oxide (ITO). The resistivity of the hydrogel is almost identical to that of water containing the same concentration of NaCl when the hydrogel is not stretched and increases when the hydrogel is stretched (Fig. 4A). For a conductor whose resistivity is independent of deformation, the re-

sistance of the conductor increases with stretch as $R/R_0 = \lambda^2$, where R_0 is the resistance before the conductor is stretched λ times its initial length. This prediction closely approximates the measured resistance-stretch curve for the ionic hydrogel (Fig. 4B). When SWNTs on VHB are stretched, the resistance increases far faster than the prediction of the square law, indicating that the resistivity of the SWNTs is increased greatly by the stretch. An 11-mm-thick hydrogel containing 5.48 M NaCl shows 98.9% average transmittance in the visible range (Fig. 4C), corresponding to a transmittance of 99.99% for a 100- μ m-thick hydrogel used in constructing actuators and loudspeakers. Among all conductors of electricity,

these hydrogels show the highest transmittance (Fig. 4D).

The diversity of ionic conductors creates a large pool of candidates for applications. Hydrogels are easy to make and inexpensive, ideal for demonstrating conceptual designs and for fabricating devices that require biocompatibility. In higher organisms, it is ionic conductors that transmit *in vivo* information. This characteristic offers the potential to build nondamaging interfaces between ionic/biological and ionic/electronic signals. Although the loss of water from hydrogels can be an issue in some applications, the rate of evaporation may be reduced by encapsulation. Also, ionic liquids are nonvolatile electrolytes and can be used as conductors for large-strain, high-speed dielec-

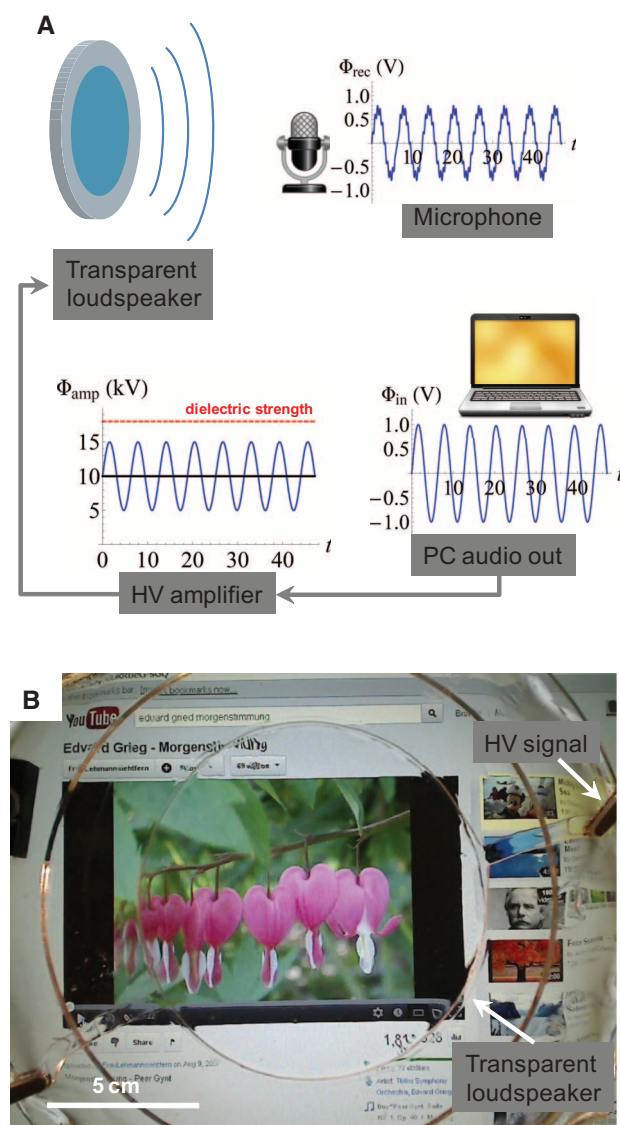
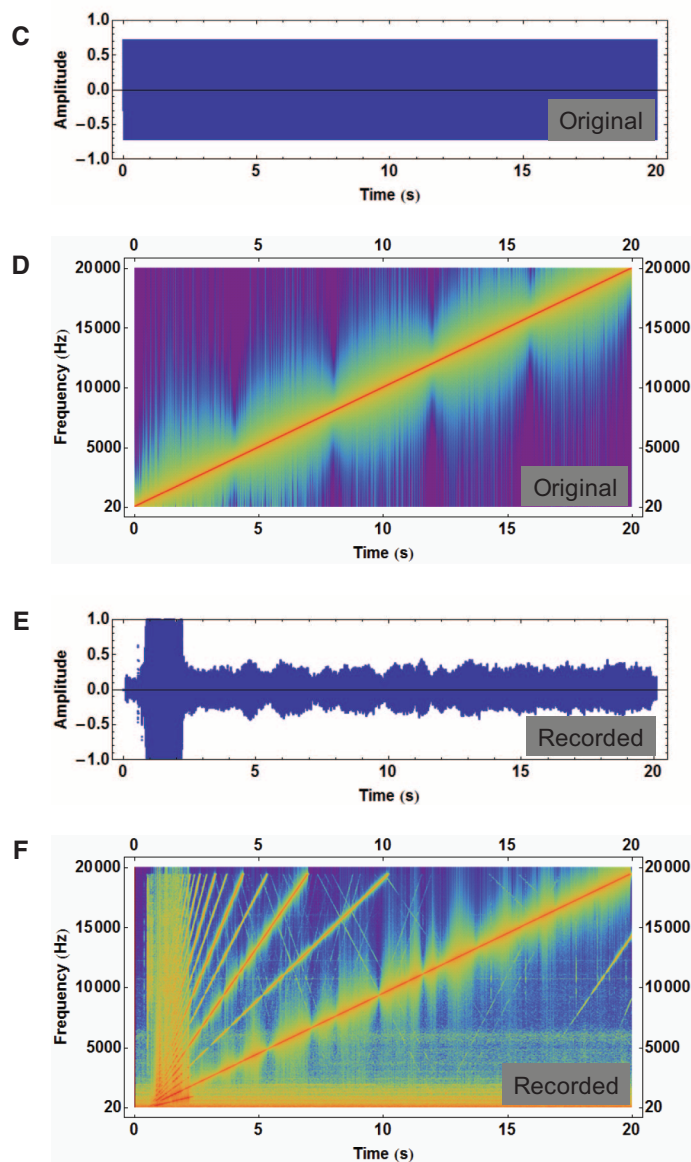


Fig. 3. Transparent loudspeaker capable of producing sound across the entire audible range. (A) The voltage signal from the audio output of a laptop was fed through a high-voltage amplifier to the loudspeaker. The loudspeaker transformed the voltage signal into sound, which was recorded with a microphone. (B) The screen of the laptop was visible through



the loudspeaker. Amplitude (C) and spectrogram (D) of a test signal with constant amplitude and a linear sine sweep of frequency from 20 Hz to 20 kHz. The colors correspond to the intensities of frequency, with warmer colors indicating higher intensity. Amplitude (E) and spectrogram (F) of the sound generated by the loudspeaker.

tric elastomer actuators (fig. S6). Furthermore, it will be interesting to explore polyelectrolytes, in which charged polymers covalently cross-link into networks, and counterions are mobile.

Replacing electronic conductors with ionic conductors raises further scientific questions. For example, various types of conductors may lead to different characteristics of charge transport through the dielectric. We have measured charge leakage through VHB when three types of conductors are used: carbon grease, a hydrogel, and an ionic liquid. The three types of conductors give a comparable time scale for leakage for the chosen experimental conditions (fig. S7). As a second example, we used thin lines of ionic conductors, hydrogels and ionic liquids, to link active regions of devices to the copper electrodes. Our calculations indicate that ionic conductors can serve as high-speed, long-distance interconnects (fig. S8). As a third example, the lifetime of a device depends on the electrolyte, the dielectric, and the electrode. However, finding ideal combinations of materials for long lifetimes is a large undertaking beyond the scope of this paper.

Our design of layered electrolytic and dielectric elastomers differs from existing transducers in which ionic conduction plays essential roles, such as actuators made of carbon nanotubes and conducting polymers in electrolytes (25, 26), actuators made of ionic polymer-metal composites (27), and resistive strain sensors made of elastomeric ionic hydrogels (28). Our design introduces

a dielectric in series with an electrolyte, and the small capacitance of the dielectric enables high-speed and large-strain actuation. Layered electrolytes and dielectrics also appear in one set of electrowetting devices, but the electrolytes are liquids, and the dielectrics do not deform (29).

Stretchable ionics offer new opportunities for designers of soft machines. Dielectric elastomers have been paired with electronic conductors to make transducers in robotics, bioelectronics, and energy harvesting (30). Pairing dielectric elastomers with ionic conductors will substantially expand the scope of applications, leading to devices such as tunable optics and localized haptics with transparent ionic conductors placed in the path of the light. Transparent loudspeakers might be attached to windows to achieve active noise cancellation (22). The ultrahigh transparency and compliance of the ionic conductors will enable transparent devices of multilayered electrolytic and dielectric elastomers. To scale up the active area of a device, one can enlarge the electrode/electrolyte interface by using porous electrodes, such as those used in supercapacitors. This paper focuses on the use of ionic conductors in devices operating at high speed and under high voltage, but the layered electrolytic and dielectric elastomer also works for applications that require low voltage or low frequency. When stretched mechanically, the layered material increases area and reduces thickness, so that its capacitance increases. This characteristic will enable transparent sensors

operating at low voltage, capable of measuring strains over a large range, and conformal to soft tissues.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/341/6149/984/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S8
References (31–43)
Movies S1 to S4

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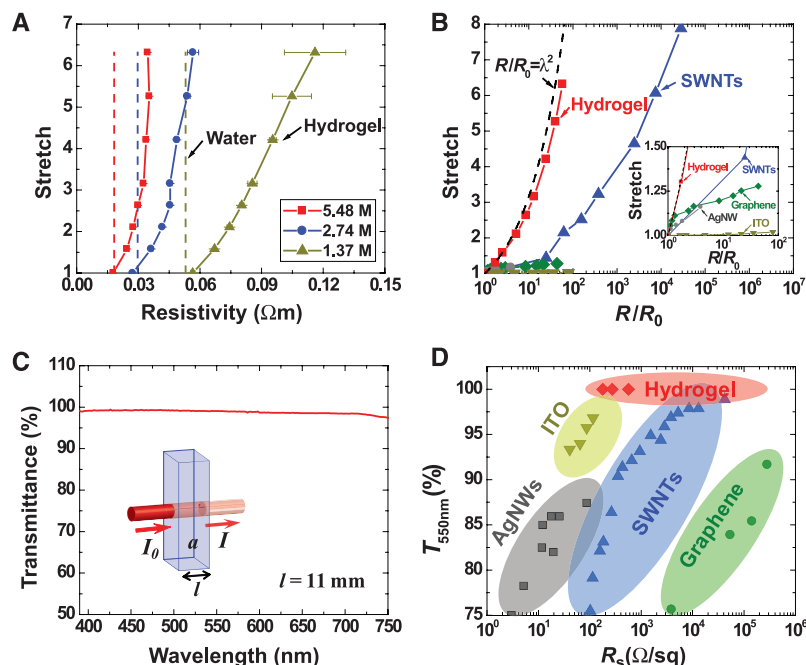


Fig. 4. Performance of ionic and electronic conductors. (A) Electrical resistivities of hydrogels of several concentrations of NaCl were measured as functions of stretch and compared with the resistivities of water containing the same concentrations of NaCl. Error bars show standard deviation; sample size $N = 3$. (B) Stretch was plotted against normalized resistance for ITO (13), AgNWs (12), graphene (9), and hydrogel (this work). (Inset) Details in the small stretch region. (C) An 11-mm-thick polyacrylamide hydrogel containing 5.48 M NaCl shows 98.9% average transmittance in the visible range. (D) Transmittance (at 550 nm) is plotted against sheet resistance for ITO (12), AgNWs (11), SWNTs (8), graphene (10), and hydrogels (100- μ m thickness, this work).

Reaction of O_2 with Subsurface Oxygen Vacancies on TiO_2 Anatase (101)

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Oxygen (O_2) adsorbed on metal oxides is important in catalytic oxidation reactions, chemical sensing, and photocatalysis. Strong adsorption requires transfer of negative charge from oxygen vacancies (V_O s) or dopants, for example. With scanning tunneling microscopy, we observed, transformed, and, in conjunction with theory, identified the nature of O_2 molecules on the (101) surface of anatase (titanium oxide, TiO_2) doped with niobium. V_O s reside exclusively in the bulk, but we pull them to the surface with a strongly negatively charged scanning tunneling microscope tip. O_2 adsorbed as superoxo (O_2^-) at fivefold-coordinated Ti sites was transformed to peroxy (O_2^{2-}) and, via reaction with a V_O , placed into an anion surface lattice site as an $(O_2)_O$ species. This so-called bridging dimer also formed when O_2 directly reacted with V_O s at or below the surface.

Molecular O_2 interacts weakly with fully oxidized metal oxides. When excess electrons are present, it adsorbs as an anion in either superoxo (O_2^-), peroxy (O_2^{2-}), or

dissociated ($2 \times O^{2-}$) form. The negative charge can be provided by intrinsic defects [e.g., oxygen vacancies (V_O s) or cation interstitials] in a reduced oxide, by doping, or through photoexcitation. Such adsorbed oxygen plays a key role in several technological processes; for example, in oxidation reactions promoted by heterogeneous catalysts, in gas sensors, or in photocatalysis (1, 2). Because of the complexity of technical materials, multiple oxidation states, and the background of lattice oxygen, a molecular-level understanding of O_2 adsorption is just emerging (3, 4). Scanning

tunneling microscopy (STM) studies on macroscopic, single-crystalline samples with flat surfaces and controlled defects allow a direct view of adsorbed species. In conjunction with density functional theory (DFT) calculations, these studies provide fundamental insights into a rich surface chemistry (3–9). However, experimental studies have not focused on anatase that makes up nanophase titania, nor have they addressed the role of subsurface V_O s that are prevalent in this material (10, 11). Here, we show that manipulations with the scanning tunneling microscope tip can be used to (i) locally create surface V_O s, (ii) alter the charge state of adsorbed O_2 , and (iii) react adsorbed O_2 with subsurface V_O s to produce an interstitial (O_2)_O species (or bridging O_2 dimer) that has been predicted theoretically.

Our sample was an anatase mineral single crystal, naturally doped with 1.1 atomic % niobium [see the supplementary materials (12)], an efficient electron donor. Although the thermodynamically stable rutile phase [especially the (110) surface of single crystals] has been extensively used as a model surface (3, 4, 13), nanophase TiO_2 , which is often used in applications, usually consists of anatase (14). Anatase is also frequently referred to as photocatalytically more active, and several studies (15–17) have shown that substantial amounts of O_2 are present on the surface of photoirradiated anatase, but not on rutile. This could be caused by the different response to

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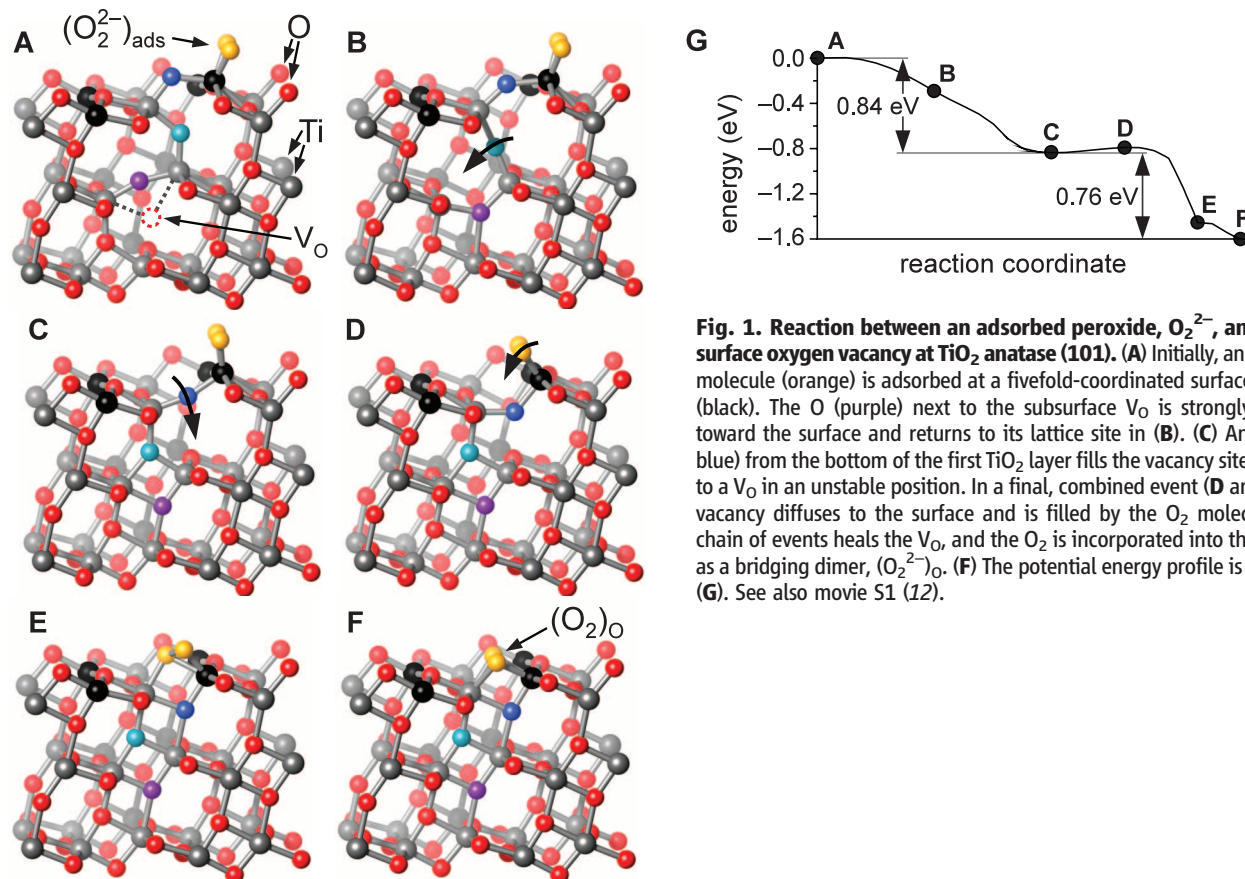


Fig. 1. Reaction between an adsorbed peroxide, O_2^{2-} , and a subsurface oxygen vacancy at TiO_2 anatase (101). (A) Initially, an $(O_2^{2-})_{ads}$ molecule (orange) is adsorbed at a fivefold-coordinated surface Ti atom (black). The O (purple) next to the subsurface V_O is strongly relaxed toward the surface and returns to its lattice site in (B). (C) An O (light blue) from the bottom of the first TiO_2 layer fills the vacancy site, leading to a V_O in an unstable position. In a final, combined event (D and E), the vacancy diffuses to the surface and is filled by the O_2 molecule. This chain of events heals the V_O , and the O_2 is incorporated into the surface as a bridging dimer, $(O_2^{2-})_O$. (F) The potential energy profile is shown in (G). See also movie S1 (12).

photoexcitation, notably by long-lived photoexcited electrons present in anatase but not in rutile (18), which provide the charge for oxygen adsorption, or by the specific surface properties of anatase (2, 19).

In contrast to TiO₂ rutile (110), V_Os are not stable at the surface of anatase (10, 11). When surface V_Os are created by electron bombard-

ment, they move into the bulk at temperatures as low as 200 K (11). In DFT calculations for an anatase slab with a subsurface V_O (which provides negative charge), O₂ adsorbs molecularly at fivefold-coordinated surface (Ti_{5c}) atoms (Fig. 1A) as a peroxo (O₂²⁻) and a superoxo (O₂⁻) species at low and high coverages (20), respectively. Ti interstitials, which lead to dissociation of O₂ at

rutile (110) (5), have the same effect on anatase (101) (21).

A peroxide (O₂²⁻)_{ads} adsorbed in the vicinity of a subsurface V_O (Fig. 1A) induces substantial structural relaxation, suggesting the existence of an energetically more favorable configuration. In first-principles molecular dynamics (FPMD) simulations (at 220 K), we observed the cascade of events shown in Fig. 1, B to E. In the resulting structure, the O₂ takes the position of a twofold-coordinated O (O_{2c}) atom in a bridging, side-on η^2 configuration; in Kröger-Vink notation, we refer to this species as an (O₂)_O. The (O₂)_O retains a bond length of 1.46 Å, characteristic of O₂²⁻ (20). This species (often referred to as interstitial or bridging O₂) has been predicted consistently in theoretical calculations of oxygen in or on anatase (22, 23) and has been proposed to be an important intermediate in the photocatalytic splitting of H₂O.

If such an (O₂)_O species exists, it should also form when an O₂ directly reacts with a surface V_O. To test this hypothesis, we prepared surface V_Os on anatase (101) (Fig. 2). In previous work, we created such V_Os by bombarding TiO₂ with electrons (11, 24). Here, we find that identical defects can be generated by the scanning tunneling microscope tip. Figure 2 shows STM images after scanning with high bias voltage and tunneling current. The bright spots within a well-defined area (Fig. 2A) are the same V_Os as

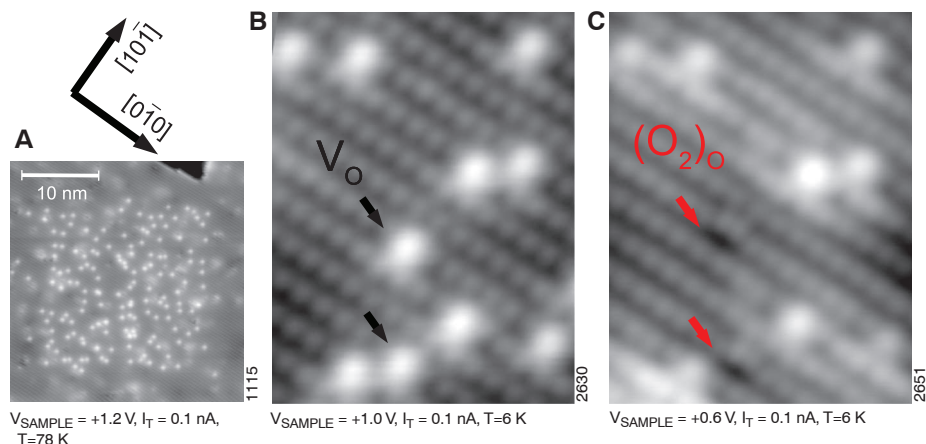


Fig. 2. Interaction of O₂ with surface oxygen vacancies (V_O) on TiO₂ anatase (101). In reduced anatase, V_Os are normally present within the bulk. In (A), these were pulled to the surface locally by scanning the center with a high sample bias of +5.2 V. In the high-resolution images of the same area before (B) and after (C) dosing 0.15 Langmuir (L) O₂ at 45 K, the arrows point out V_Os that reacted with O₂, forming the (O₂)_O configuration shown in Fig. 1F. I_T, tunneling current; T, temperature.

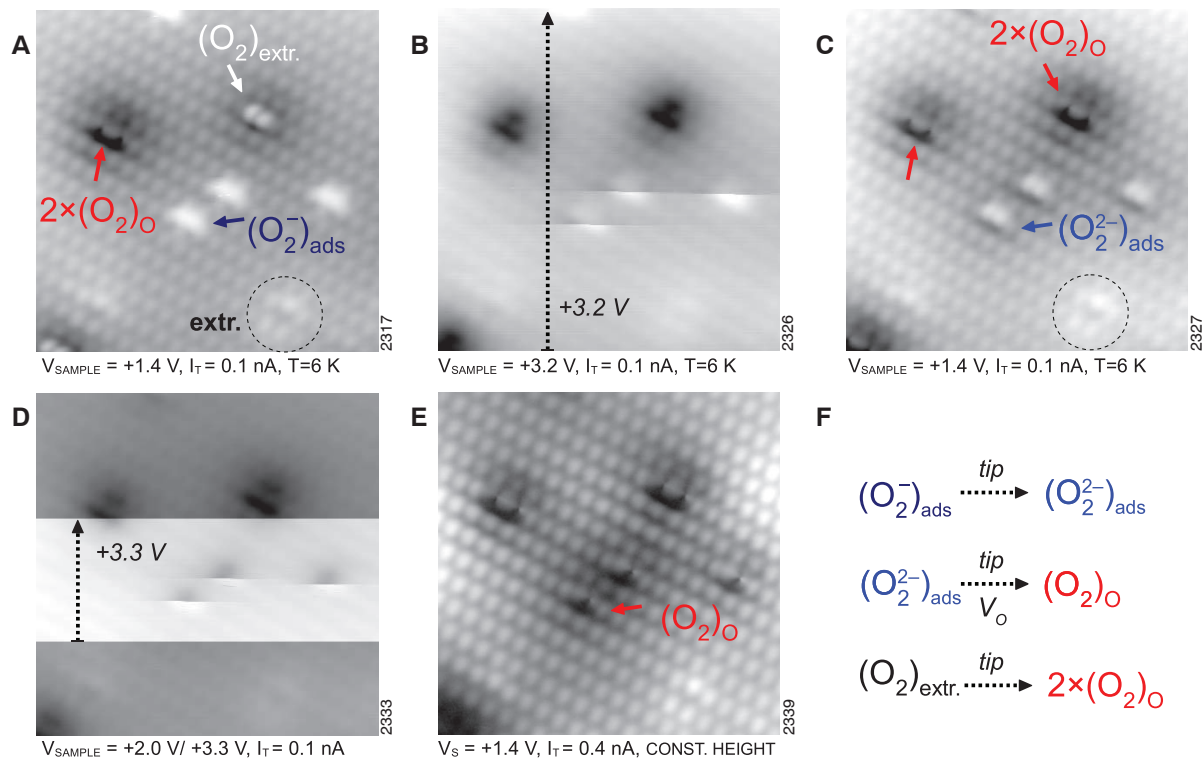


Fig. 3. Scanning tunneling microscope tip-induced conversion of adsorbed O₂. Sequence of STM images of the same area of an anatase (101) surface after exposure to 20 L O₂ at 105 K. (A) Various configurations of O₂: at regular sites, (O₂)_{ads}; at the position of lattice oxygen, (O₂)_O; and at the dopants, (O₂)_{extr}. The dopants are likely Nb; one of them is marked with a dashed

circle. After increasing the sample bias voltage to +3.2 V (B), the (O₂)_{extr} is converted to two (O₂)_O species and the (O₂)_{ads} to an intermediate species, likely a peroxo ion, (O₂²⁻)_{ads} (C). An additional scan with V_{sample} = +3.3 V (D), converts each (O₂²⁻)_{ads} into one (O₂)_O species (E). (F) Overview of the tip-induced processes.

the ones generated by electron irradiation (11). After dosing with O_2 (Fig. 2), some of the V_{OS} were replaced by double spots located at O_{2c} sites. Their appearance agrees well with calculated STM images of the $(O_2)_O$ configuration (fig. S4). The $(O_2)_O$ species did not form at temperatures below 20 K, but it readily appeared when O_2 was dosed above 40 K, with a sticking coefficient S near unity. This result roughly fits the DFT-derived activation energy of 47 meV (see Fig. 1G).

When we dosed a surface without such artificially created surface V_{OS} , we also see the same $(O_2)_O$ configuration, albeit quite rarely (see supplementary materials). Most often, we observed two different O_2 species (Fig. 3). One of them, denoted $(O_2)_{extr}$, is related to the Nb dopant that preferably replaces a surface Ti_{6c} atom (fig. S2 and table S2). These dopants are distributed unevenly at the surface, with an average concentration of 0.5% of a monolayer [(ML), defined as the density of surface Ti_{5c} atoms] and a local variation between 0.1 and 1% ML. In STM, these impurities exhibit a typical triangular shape (Fig. 3A and fig. S1). Upon O_2 exposure at 105 K, bright dimers were found at the position of these extrinsic dopants; they adsorbed with $S \approx 0.1$. In DFT calculations (fig. S3), the adsorption of an O_2 molecule at a Ti_{5c} atom is preferred by 0.12 to 0.15 eV when next to a Nb_{6c} atom.

In addition to $(O_2)_{extr}$, a bright species, labeled $(O_2)_{ads}$, formed with $S \approx 10^{-4}$ to 10^{-2} when anatase (101) was exposed to O_2 at 100 K. (The higher values were observed on a more reduced crystal.) The $(O_2)_{ads}$ is located at regular Ti_{5c} surface atoms. Its concentration increases linearly with O_2 exposure (fig. S5). The highest coverage observed was 5% ML. For higher exposures, STM imaging becomes difficult; the saturation coverage could be considerably higher.

The $(O_2)_{ads}$ can be converted into other species with the scanning tunneling microscope tip. Figure 3A shows several $(O_2)_{ads}$ and $(O_2)_{extr}$. During scanning at $V_{sample} = +3.2$ V (Fig. 3B), horizontal streaks indicate that the adsorbates were modified by the presence of the tip. (The slow scan direction was from bottom to top.) The $(O_2)_{ads}$ became more dimerlike (Fig. 3C), and the dark contours indicate that this new species carried a more negative charge. When subjecting these intermediate species to a slightly higher voltage (Fig. 3D), a second conversion took place; see Fig. 3E. The resulting species is the $(O_2)_O$ that also formed when an O_2 molecule directly reacted with a surface V_O . (See supplementary materials for more experimental evidence.) Also note that the $(O_2)_{extr}$ was already converted into two $(O_2)_O$ species during the first high-voltage scan (Fig. 3, B and C) and that two $(O_2)_O$ were always produced per one $(O_2)_{extr}$, whereas only one $(O_2)_O$ species resulted from each $(O_2)_{ads}$ (Fig. 3F). For example, the two neighboring $(O_2)_O$ configurations indicated in the initial image (Fig. 3A) resulted from previously scanning an $(O_2)_{extr}$ at high bias. The tip-induced conversion was re-

producible on different samples and with different tips at a minimum V_{sample} of $+3.3 \pm 0.1$ V.

From these results, we derive a complete picture of the reaction of O_2 with a reduced TiO_2 anatase (101) surface. When O_2 impinges on the cold (~ 100 K) sample, it is weakly adsorbed. Stronger adsorption occurs when electrons are transferred from the surface to the molecule. The Nb^{5+} dopants with nearby electrons are populated first, resulting in $(O_2)_{extr}$ species. A molecule can also extract an electron from TiO_2 at a terrace site to form an $(O_2)_{ads}$. Adsorbed O_2 species have a high binding energy (20) and are immobile in STM.

Although $(O_2)_O$ species form on as-dosed surfaces with subsurface V_{OS} , their concentration is low. The spontaneous healing process depicted in Fig. 1 happens rarely. V_{OS} are more stable in the subsurface region than on the surface by ~ 0.4 eV (10). The activation energy for a V_O to hop from the surface to the first subsurface layer ranges from 0.6 to 1.2 eV, and the barrier for the reverse process is higher by ~ 0.4 eV (11). In the bulk, V_{OS} diffuse with a much lower barrier of ~ 0.2 eV; thus, they tend to avoid the surface. Although an adsorbed, negatively charged O_2 reverses the energy balance (Fig. 1), the bulk V_{OS} seldom come close enough to the seldedge for the healing process to take place.

We pulled V_{OS} to the adsorbate-free surface with a sufficiently negative scanning tunneling microscope tip. Figure S10 shows that this process is clearly field-induced. The field likely reaches into the semiconductor (tip-induced band-bending) and pushes away electrons that are more or less localized around the V_O . This ionizes and destabilizes the vacancy, similar to the effect triggered by draining of electrons via localization at the O_2^{2-} in the FPMD simulations. The threshold bias voltage for this process is 4.5 eV; the process becomes efficient at 5.2 V. The field-induced migration of intrinsic defects within TiO_2 is already used in novel memory devices, but the nature of the mobile species is controversial (25). Our results show that V_{OS} in TiO_2 can be manipulated by high electric fields.

The tip-induced transformations of the adsorbed O_2 species are summarized in Fig. 3F. We propose that, initially, superoxide $(O_2)^-_{ads}$ forms at regular Ti_{5c} sites. These are transformed into peroxide ions; the increase in band-bending observed after the first tip-induced conversion step suggests that the O_2 becomes more negatively charged. A similar, thermally activated transformation occurs if the sample is heated to temperatures between 200 and 300 K (fig. S11). DFT predicts similar adsorption configurations for $(O_2)^-_{ads}$ and $(O_2)^{2-}_{ads}$, except for a slightly longer bond length in the peroxide ion (1.33 versus 1.48 Å) (20). By hybrid functional calculations (26), we found a barrier of ~ 0.3 eV to transform $(O_2)^-_{ads}$ into the more stable $(O_2)^{2-}_{ads}$ species. Once the $(O_2)^{2-}_{ads}$ is formed, somewhat higher bias voltages (and/or prolonged exposure to the field) help the adsorbed molecule to merge

with a subsurface V_O , resulting in the bridging dimer at a lattice site, $(O_2)_O$. For $(O_2)_{extr}$ near the Nb impurity, the situation is different. $(O_2)_{extr}$ is dissociated by the tip with a threshold voltage of 1.6 to 2.5 V, smaller than the 3.3 ± 0.1 V needed for the $(O_2)_{ads} \rightarrow (O_2)_O$ conversion. Each of the resulting adatoms merges with an O_{2c} atom to form an $(O_2)_O$ species, consistent with the DFT predictions (23).

Our results clearly show the importance of subsurface O vacancies in TiO_2 anatase. They also exemplify how the electric field—for example, from a scanning tunneling microscope tip—can be used as an effective tool to control the charge state of photocatalytically active species. The field can induce transformations of adsorbed O_2 that, by merging with a subsurface V_O , ultimately lead to the formation of the $(O_2)_O$ interstitial. This is by far the most stable O_2 species on the anatase surface. $(O_2)_O$ is also an important intermediate in the photooxidation of water (23), which suggests that it could contribute to the higher photocatalytic activity of anatase relative to rutile. Thus, manipulating and controlling the $(O_2)_O$ interstitial might be a key to furthering the development of more active O-rich TiO_2 photocatalysts for water oxidation (27).

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Supplementary Materials

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Materials and Methods

Supplementary Text
Figs. S1 to S11
Tables S1 and S2
References (28–30)
Movies S1 and S2

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Electron Acceleration in the Heart of the Van Allen Radiation Belts

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The Van Allen radiation belts contain ultrarelativistic electrons trapped in Earth's magnetic field. Since their discovery in 1958, a fundamental unanswered question has been how electrons can be accelerated to such high energies. Two classes of processes have been proposed: transport and acceleration of electrons from a source population located outside the radiation belts (radial acceleration) or acceleration of lower-energy electrons to relativistic energies in situ in the heart of the radiation belts (local acceleration). We report measurements from NASA's Van Allen Radiation Belt Storm Probes that clearly distinguish between the two types of acceleration. The observed radial profiles of phase space density are characteristic of local acceleration in the heart of the radiation belts and are inconsistent with a predominantly radial acceleration process.

Radial diffusion of geomagnetically trapped electrons occurs continuously in Earth's time-varying magnetic field. Early theo-

ries of the formation of the Van Allen radiation belts focused on betatron and Fermi acceleration processes that act when electrons are transported from the outer magnetosphere where magnetic fields are weak (<100 nT) to the radiation belts, in the inner magnetosphere, where the magnetic fields are strong ($1, 2$). The single-point measurements and low time resolution of early satellite observations suggested that radial diffusion could generally explain the equilibrium structure of the radiation belts and its evolution on the longer time scales (days to weeks) revealed in early satellite observations. In the 1990s, a growing network of satellites provided multipoint measurements with temporal and spatial resolutions, revealing com-

plex structure and rapid dynamics that were difficult to explain with conventional theory.

In January 1997, a solar coronal mass ejection produced a strong geomagnetic storm and a dramatic intensification of radiation belt electron fluxes at energies up to several MeV ($3, 4$). Comparison of the dynamics at geosynchronous orbit [$\sim 6.6R_E$ (R_E is Earth's radius, 6372 km) or 42,000 km geocentric distance] and in the heart of the electron belt ($\sim 4.2R_E$ or 27,000 km) showed that the intensification of relativistic electron fluxes occurred first in the heart of the belts and on extremely rapid time scales (~ 12 hours) and only later and more slowly at higher altitudes. In contrast with radial diffusion theory, these observations strongly suggested an energization process operating locally in the heart of the radiation belts (3). A leading candidate for that process was proposed to be acceleration by resonant interactions between radiation belt electrons and naturally occurring electromagnetic very low frequency (VLF) ($\gtrsim 1$ kHz, i.e., radio) waves ($5-10$). However, around the same time other observations (11) showed a strong correlation between radiation belt electron enhancements and the power in global ultralow frequency (ULF) waves, which are enhanced during geomagnetic storms. Subsequent studies suggested that rapid enhancements of the radiation belts could be explained by acceleration from rapid, time-varying radial diffusion driven by the strong ULF field fluctuations ($12-17$).

Measurements of electron flux (electrons $\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{MeV}^{-1}$) cannot distinguish between local acceleration and acceleration by radial transport because both can produce radial peaks in electron flux. However, phase space density, which is the

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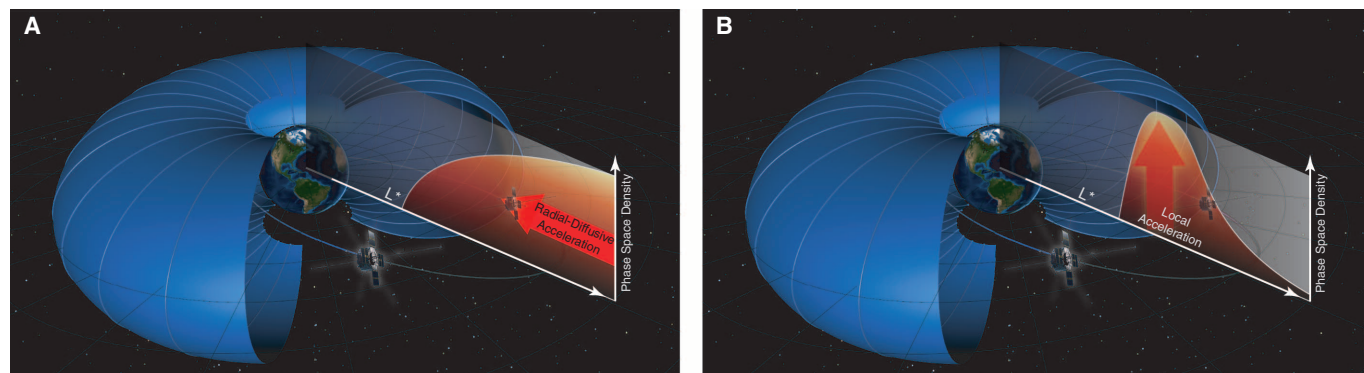


Fig. 1. The geometry of the radiation belts and RBSP orbits shown to scale. The positions of the RBSP satellites at 07:57 UT on 9 October are indicated. Also shown, in a cut-away view, is a single drift shell for electrons with 15° equatorial pitch angles starting at the position of RBSP-A at a radial

distance $L^* \approx 4.2$. Representative magnetic field lines are plotted in light blue between the northern and southern magnetic mirror points. The radial profiles of phase space density expected from radial-diffusive acceleration (**A**) and local wave-particle acceleration (**B**) are indicated schematically.

electron flux divided by the square of the momentum, does show unique signatures for local versus radial acceleration when expressed as a function of “magnetic coordinates.” NASA’s recently launched Van Allen Radiation Belt Storm Probes (RBSP) mission was designed to make the measurements needed to distinguish whether local or radial acceleration is the primary driver behind radiation belt electron acceleration events (18). Here, we report on the radial profiles of phase space density observed during the first major radiation belt enhancement event of the RBSP mission.

In Earth’s magnetic field, the motion of electrons is constrained to “drift shells” where electrons bounce between northern and southern magnetic mirror points and drift azimuthally around Earth (Fig. 1). To distinguish between local and radial acceleration, we must express phase space density not as it is measured—as a function of energy, pitch angle, and position—but rather as a function of the magnetic coordinates, μ , K , and L^* ,

that constrain electron motion (figs. S1 and S2). For radiation belt electrons, the quantity L^* defines their radial location, as measured from the center of Earth. Radial diffusion moves electrons in L^* while conserving the quantities μ and K . Because it is a stochastic process, diffusion moves electrons from regions of higher to lower phase space density. Therefore, enhanced radial diffusion from a source population at high L^* can increase the phase space density at lower L^* , but the gradients will still exhibit a monotonic decrease from the source or, equivalently, a monotonic increase with increasing L^* (Fig. 1A). In contrast, local acceleration processes keep an electron’s position (L^*) essentially constant while increasing its energy. Therefore, local acceleration processes produce increases in phase space density over a limited range of L^* , which, if sufficiently strong, will lead to a local peak in the radial profile with negative radial gradients at higher L^* (Fig. 1B).

Beginning with the January 1997 radiation belt electron event, studies have analyzed the radial profiles of phase space density and have provided growing evidence for local acceleration (4, 19–22). However, those studies were limited by a number of factors, including detector limitations, high backgrounds from penetrating radiation, poor energy coverage or resolution, limitations imposed by the satellite inclination or orbital period, and limited radial coverage (23). The RBSP mission was designed specifically to overcome those limitations by providing measurements near the magnetic equator, with broad and continuous energy coverage and rapid radial cuts through the heart of the radiation belts, and simultaneous measurements from spatially separated satellites (24). The two RBSP satellites were launched on 30 August 2012 into a near-equatorial, elliptical orbit with apogee at $5.7R_E$.

On 9 October 2012, the twin RBSP satellites measured an intense relativistic electron acceleration

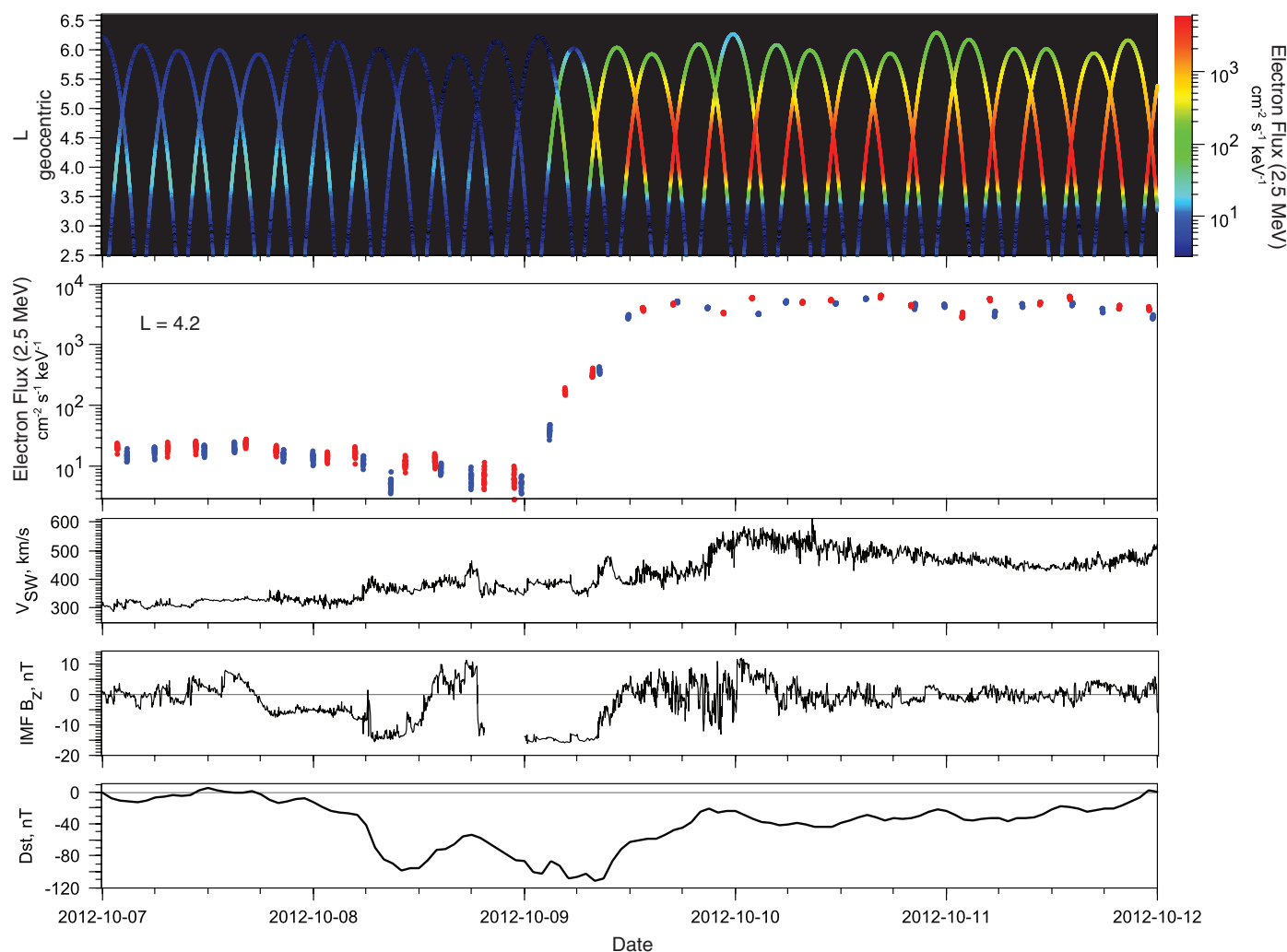


Fig. 2. An overview of the October 2012 geomagnetic storm and radiation belt electron event. The top graph shows the flux (intensity) of 2.5 MeV electrons from the MagEIS magnetic spectrometer (30) on NASA’s twin RBSP satellites. The second graph shows the fluxes at a radial distance of $L = 4.2$. The bottom three graphs show solar wind speed (V_{sw}), the

interplanetary magnetic field north-south component (IMF B_z), and the disturbance storm time index (Dst, a measure of geomagnetic storm intensity). The geomagnetic activity late on 8 October and into 9 October produced a very intense and very rapid increase in fluxes. More information on solar wind, geomagnetic, and VLF wave conditions is in the supplementary materials.

ation event (Fig. 2). Geomagnetic storms can either intensify or deplete the fluxes of MeV electrons in the outer belt (25). A storm on 1 October strongly depleted the outer electron belt (26), and electron fluxes remained exceptionally low and fairly constant until 8 October. The fluxes of outer belt electrons continued to gradually decrease until early on 9 October, when the fluxes of MeV electrons began to rapidly increase. In many radiation belt electron acceleration events, the fluxes

rise gradually over the course of a day or two. This event more closely resembles the January 1997 event in that the fluxes rose nearly three orders of magnitude in a period of less than 12 hours.

We plotted the time-dependent radial profiles of phase space density in order to look for the characteristic signatures of either radial or local acceleration (Fig. 3). In the first pass on 8 October (labeled 18:22), the RBSP-A spacecraft was outbound starting at $L^* = 3.6$ at 17:32 UT, reaching

Fig. 3. Phase space density profiles for relativistic radiation belt electrons. Profiles were measured by the Relativistic Electron-Proton Telescope instrument (31) on 8 and 9 October. Phase space density, f , is in units of $(\text{c}/\text{cm MeV})^3$ where c is the speed of light. By plotting $f(L^*)$ at fixed magnetic invariants, $\mu = 3433 \text{ MeV/G}$, and $K = 0.11 R_E G^{1/2}$, we can plot both inbound and outbound portions of the orbit for each satellite on a common basis, vastly increasing the spatial and temporal resolution relative to previous observations. We used the TS04 magnetic field model to calculate the invariants (32). Curves for RBSP-A (squares) and RBSP-B (circles) are color-coded and labeled by the time at which each satellite crossed $L^* = 4.2$. The rapid increase in phase space density in the vicinity of $L^* = 4.2$ and the slower, more delayed increase at higher L^* produce signatures of local acceleration: a peak in phase space density with positive radial gradients at lower L^* and negative radial gradients at higher L^* . The average uncertainty on the calculation of phase space densities at fixed μ and K is a factor of 1.4, and the maximum uncertainty is a factor of 2 as discussed in the supplementary materials and figs. S8 and S9.

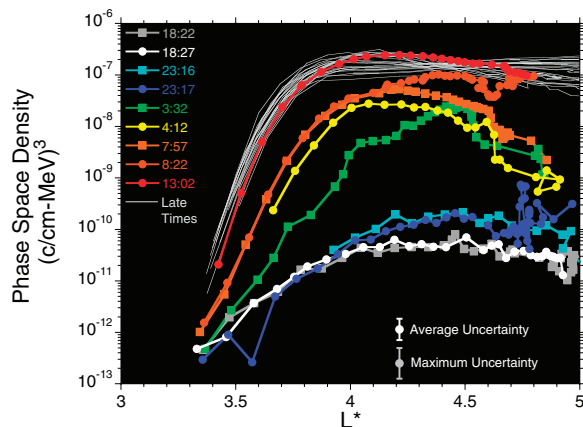
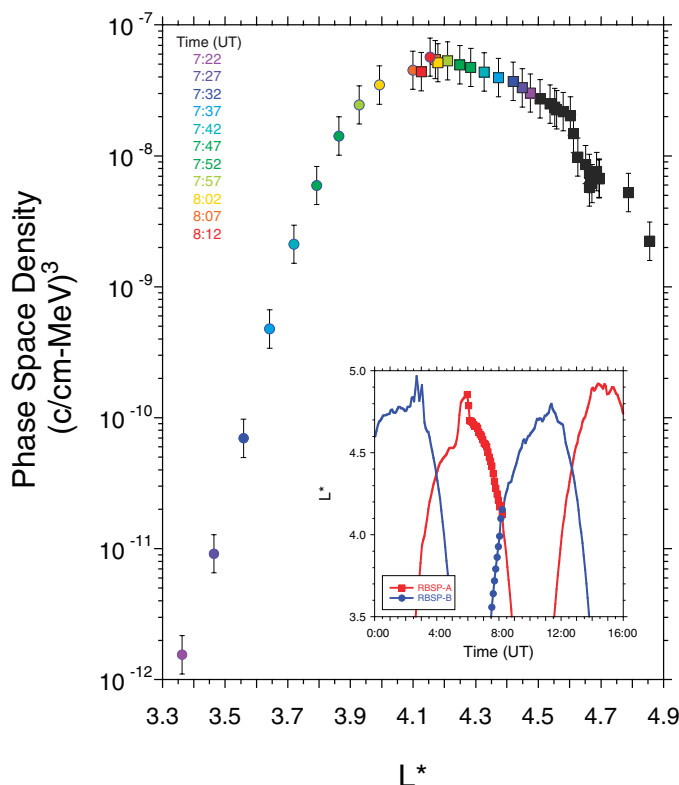


Fig. 4. Simultaneous measurements of phase space density from RBSP-A (squares) and RBSP-B (circles). Points are color-coded as a function of time when the two satellites were making simultaneous measurements of the radial gradients (07:22 to 08:12 UT). (Inset) The satellite orbits with RBSP-A inbound from apogee and RBSP-B outbound from perigee.



an apogee of $L^* = 5.2$ at 21:17 UT. Comparison with the subsequent profiles at 18:27 and 23:17 UT shows that there was little temporal evolution, and each pass can essentially be considered a snapshot of the phase space density profile.

Between the passes labeled 23:17 (8 October) and 03:32 UT (9 October), the radiation belts experienced a rapid increase in phase space density that continued for the next 10 hours. The increase in phase space density is most rapid in the heart of the radiation belts, in the vicinity of $L^* = 4.2$. In the early stages of the event, the phase space densities at higher L^* (e.g., 4.8) change only slightly to produce a pronounced peak in the radial profiles. In the passes labeled 4:12 and 7:57 UT, the radial peak in phase space density and the negative gradients at high L^* continue to grow. The pass labeled 3:32 UT is complicated because the magnetosphere was changing on time scales that are comparable to the transit of the RBSP-A satellite through the belt (figs. S6 and S7).

In the passes labeled 8:22 and 13:02 UT, the negative radial gradients at high L^* began to smooth out even as the peak phase space density increased, indicating that, in addition to local acceleration, radial diffusion also affected the radiation belt electrons by transporting them radially outward (and inward) from the newly formed peak. From 10:22 UT on 9 October through 12 October (labeled "late times"), the phase space density profiles show very little change, which is consistent with the abrupt decrease of energy input into the magnetosphere as the IMF turned northward (Fig. 2).

As noted in previous studies (18, 20, 22), single-satellite studies of phase space density gradients are subject to some spatial-temporal ambiguity because of the finite time for a single satellite to move from one L^* to another. By using simultaneous measurements of the phase space density measured at different L^* by the two RBSP satellites, we can further test whether instantaneous gradients are consistent with those seen in a finite-duration satellite pass. Starting at 05:57, RBSP-A was on the inbound leg of its orbit (labeled 7:57 in Fig. 3). RBSP-A measured a negative radial gradient with phase space densities that increased from 2.2×10^{-9} at $L^* = 4.9$ to 5.4×10^{-8} at $L^* = 4.2$. At about 07:22, RBSP-B entered the outer electron belt moving outward (orbit labeled 8:22 in Fig. 3). RBSP-B measured a positive radial gradient with phase space densities that increased from 1.6×10^{-12} at $L^* = 3.4$ to 6.5×10^{-8} at $L^* = 4.2$. Starting at 07:22, the two satellites were making simultaneous observations on opposite sides of the peak (Fig. 4). From 7:22 to 8:12 UT, RBSP-A moved inward from $L^* = 4.5$ to 4.1 and measured phase space densities went from 3.0×10^{-8} to 4.4×10^{-8} . Although this is on the order of the uncertainty in the measurements, the inward-directed gradients are a smooth continuation of the gradients measured earlier. The twin RBSP measurements confirm that the radial peak in phase space density is indeed a real spatial structure and not the result of spatial-temporal aliasing.

Numerical simulations (27) and other analyses (20) have shown that radial peaks in phase space density can in some cases be produced by radial diffusion in combination with a boundary condition at the outer edge of the belts that first increases to levels higher than the observed peak at lower L^* then decreases to levels below it. The shortness of the time between subsequent RBSP apogee passes provides stringent constraints on such a scenario but does not disprove it. Comparison between RBSP and geosynchronous phase space densities from five geosynchronous satellites that orbit outside RBSP's apogee and provide near-continuous monitoring of the outer boundary of the radiation belts confirms the negative radial gradient at high L^* , the lack of a potential source population at the outer boundary, and the necessity of an internal local acceleration process (fig. S10).

Although it is possible that radial acceleration may dominate in other relativistic acceleration electron events (28), the RBSP measurements of phase space density profiles on 8 and 9 October show signatures of local acceleration by wave-particle interactions in the heart of the radiation belts (29). The entire acceleration took place over a period of ≈ 11 hours between 23:17 UT on 8 October and 13:02 UT on 9 October. The primary acceleration was centered at $L^* \approx 4$ with evidence of acceleration observed between $L^* = 3.5$ to 4.5.

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Supplementary Materials

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A Uranian Trojan and the Frequency of Temporary Giant-Planet Co-Orbitals

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Trojan objects share a planet's orbit, never straying far from the triangular Lagrangian points, 60° ahead of (L4) or behind (L5) the planet. We report the detection of a Uranian Trojan; in our numerical integrations, 2011 QF₉₉ oscillates around the Uranian L4 Lagrange point for >70,000 years and remains co-orbital for ~ 1 million years before becoming a Centaur. We constructed a Centaur model, supplied from the transneptunian region, to estimate temporary co-orbital capture frequency and duration (to a factor of 2 accuracy), finding that at any time 0.4 and 2.8% of the population will be Uranian and Neptunian co-orbitals, respectively. The co-orbital fraction ($\sim 2.4\%$) among Centaurs in the International Astronomical Union Minor Planet Centre database is thus as expected under transneptunian supply.

During 2011 and 2012, we used the Canada-France-Hawaii Telescope to perform a 20-square-degree survey designed to de-

tect trans-Neptunian objects (TNOs) and objects between the giant planets with apparent r-band magnitude $m_r < 24.5$ and track all detections for up to 17 months. The project was accurately calibrated (I) to constrain the size and orbital parameter distributions of populations resonant with Neptune. Constraining the distribution of these populations is essential, as they in turn set constraints on models of the evolution of the outer solar system.

As part of this survey, we detected 2011 QF₉₉ (2) at a heliocentric distance of 20.3 astronomical units (AU), where its apparent magnitude $m_r = 22.6 \pm 0.1$ sets its absolute magnitude at $H_r = 9.6$ ($H_g = 10.3$, assuming a typical color $g - r \approx 0.7$). This magnitude indicates that 2011 QF₉₉ is ~ 60 km in diameter, assuming a 5% albedo. As more observations constrained the orbit, it became clear that 2011 QF₉₉ was not simply a Centaur that happened to be near the distance of Uranus. Our current astrometry, consisting of 29 measurements from seven dark runs with a total arc of 419 days, indicates the following orbital elements: $a = 19.090 \pm 0.004$ AU, $e = 0.1765 \pm 0.0007$, $i = 10.811^\circ \pm 0.001^\circ$, $\Omega = 222.498^\circ \pm 0.001^\circ$, $\omega = 287.51^\circ \pm 0.11^\circ$, and $T = 246\,4388 \pm 11$ JD. Here, a , e , i , Ω , ω , T are the osculating J2000 barycentric semimajor axis, eccentricity, inclination, longitude of ascending node, argument of pericenter, and Julian day of pericenter. The low eccentricity along with a semimajor axis similar to that of Uranus ($a_U \approx 19.2$ AU) indicated that 2011 QF₉₉ might be a Uranian co-orbital. Co-orbital bodies are in the 1:1 mean-motion resonance with a planet (thus having the same orbital period) and a librating (oscillating) resonant angle $\phi_{11} = \lambda - \lambda_{\text{Planet}}$. Here, λ is the mean longitude, which is the sum of Ω , ω , and the mean anomaly. ϕ_{11} roughly measures how far ahead in orbital phase the object

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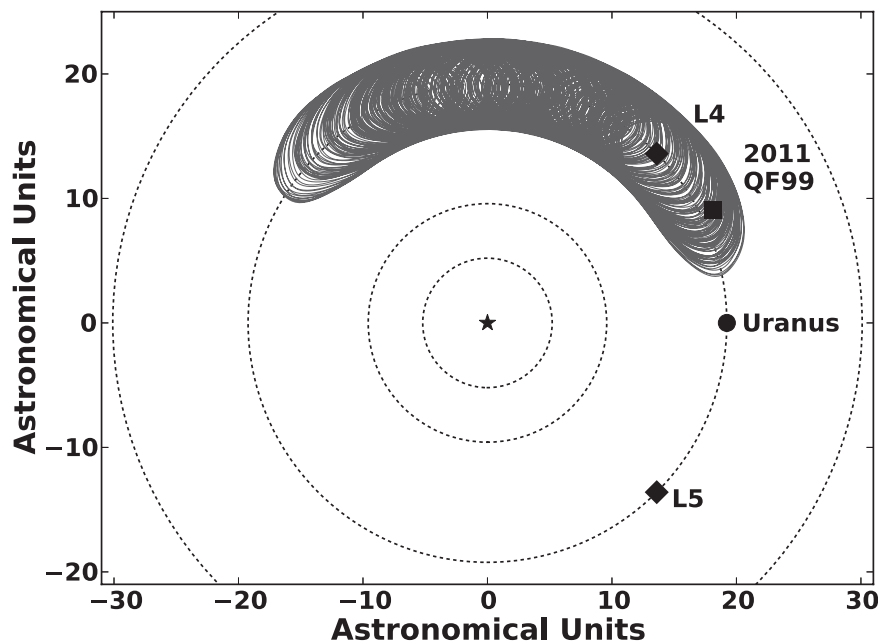


Fig. 1. The motion of 2011 QF₉₉. Shown here is the best-fit trajectory of 2011 QF₉₉, from its current position (square) to 10 libration periods (59 ky) into the future. The co-ordinate frame co-rotates with Uranus (on the right), and dotted circles show the semimajor axis of the giant planets. Diamonds denote the L4 (upper) and L5 (lower) Lagrange points. The oval oscillations occur over one heliocentric orbit, whereas the angular extent around the Sun is the slower libration around L4.

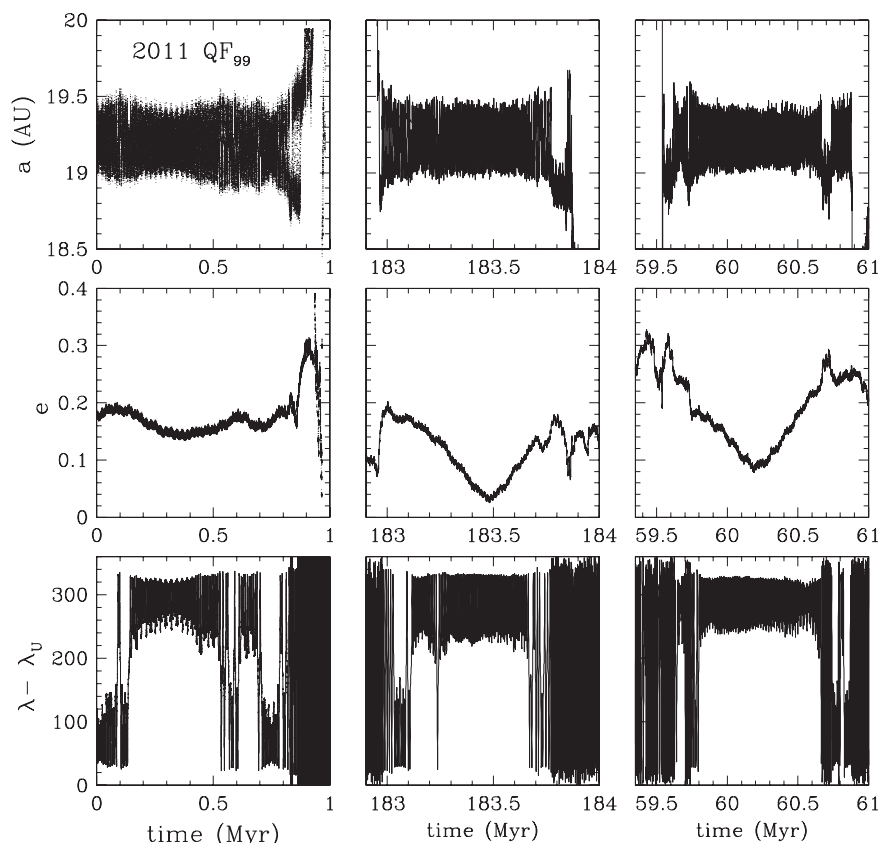


Fig. 2. Orbital evolution of temporary Uranian co-orbitals. Left column: Evolution of the nominal semimajor axis a , eccentricity e , and resonant angle $\lambda - \lambda_U$ of 2011 QF₉₉ for 1 My into the future. Center and right columns: Evolution for two temporary Uranian co-orbitals from our dynamical simulations for intervals in which their evolution is similar to that of 2011 QF₉₉, showing that Centaurs can naturally become temporarily trapped Uranian Trojans. Times are from the initial condition for the $a_0 > 34$ AU scattering orbit.

is relative to the planet. For co-orbital motion, ϕ_{11} librates around one of four values (3). Quasi-satellites librate around 0° ; in the co-orbital frame, these move like retrograde satellites, despite being outside the planet's gravitational dominance. Leading and trailing Trojans librate around L4 (60° ahead of planet) and L5 (300° ahead = 60° behind planet), respectively. Horseshoe orbits execute librations around L3 (180° from the planet) with high amplitudes that encompass the L3, L4, and L5 Lagrange points.

A short [50 thousand years (ky)] numerical integration showed that 2011 QF₉₉ is librating around the leading (L4) Lagrange point (Fig. 1). Could 2011 QF₉₉ be a primordial Trojan? Jupiter hosts a large population of Trojan asteroids stable for 5 billion years (Gy). The recently detected stable population of Neptunian Trojans are now believed to outnumber Jovians for objects with radius > 50 km (4, 5). By contrast, the Trojan regions of Saturn and Uranus are believed to be mostly unstable (6, 7) and are unlikely to host long-lived Trojans, although a few stable niches exist (7); it is unclear how migration affects the likelihood of these niches being populated (8, 9).

In longer integrations, using the 10-million year (My) time scale typically used to determine the dynamical class of outer solar system objects (10, 11), both the nominal orbit of 2011 QF₉₉ and all other orbits within the (already small) orbital uncertainties librate around the L4 Lagrange point for at least the next 70 ky (Fig. 2, left column, and fig. S1). On time scales of 100 ky to 1 My, all integrated orbits transition out of the L4 Trojan region (1), either escaping directly to scattering behavior (that is, become Centaurs) or transitioning to other co-orbital behavior before escaping and scattering away within 3 My.

We considered the possibility that the initial investigation missed a small phase-space niche, stable for 4 Gy, or that systematic errors could result in the real orbit being offset by several tens of times the nominal uncertainties. We thus integrated 10^5 test particles filling the region within $\Delta a = \pm 0.1$ AU, $\Delta e = \pm 0.004$, and $\Delta i = \pm 0.2^\circ$ of the nominal orbit for up to 0.1 Gy, until they crossed the orbit of Saturn or Neptune. All 100,000 particles were eliminated within 100 My, most within the usual 10-My stability of Centaurs (12, 13). This rejects the idea that 2011 QF₉₉ has been a Uranian Trojan for very long; it must instead be a Centaur recently temporarily trapped into L4 libration.

Temporary co-orbitals are known elsewhere in the solar system (1). In this survey, 2011 QF₉₉ was the only object with a semimajor axis within the planetary region (defined here as $a < 34$ AU to include Neptune co-orbitals but exclude the exterior stable transneptunian populations). The Canada-France Ecliptic Plane Survey (CFEPS) detected three $a < 34$ AU objects and the International Astronomical Union (IAU) Minor Planet Center (MPC) database contains 247 objects with $6 \text{ AU} < a < 34 \text{ AU}$ as of 9 July 2013. We sought to determine whether (to a factor of 3) it is reasonable

that, in a model of Centaur supply from the scattering disk, a large-enough fraction should be in resonance at any time to explain the observed discovery of 2011 QF₉₉ and other temporary co-orbitals. To address this question, we estimated the fraction of Centaurs in temporary co-orbital states with Uranus and Neptune, similar to what has been done for Earth (14) and Venus (15). Even though the scattering population is depleting with time, the co-orbital fraction does not (1). Here, “scattering objects” are those (10, 16) that experience $\Delta a > 1.5$ AU in 10 My; scattering objects with $a < 30$ AU are called “Centaurs,” whereas those with $a > 30$ AU are the “scattering disk.”

Using a model of the orbital distribution (17) of today’s scattering TNOs (1), we simulated the interactions of scattering objects with the giant planets over 1 Gy, building a relative orbital distribution for the $a < 34$ AU region (1). The simulation outputs the state vector of planets and all $a < 34$ AU particles at 300-year intervals. This output interval was chosen so that the few-thousand-year variation of the resonant argument ϕ_{11} would be well sampled (Fig. 2), allowing detection of short-term co-orbitals of the giant planets. Such a meticulous search for co-orbitals trapped from an armada of incoming scattering objects is essential to accurately estimate the trapping fraction. An earlier analysis (18) started with a sample of currently known Centaurs, which was biased toward the lowest- a Centaurs by observational selection, resulting in much lower trapping rates for Uranus and Neptune than we find.

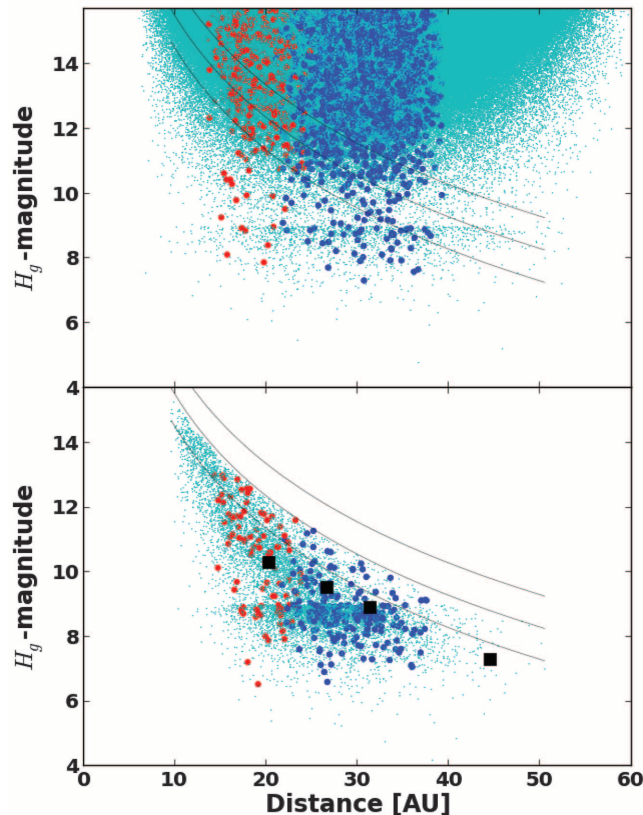
Simulated scattering objects predominantly entered the giant planet region ($a < 34$ AU) at intermediate inclinations and eccentricities, as previously shown (13, 19). After analyzing the particle histories to find co-orbital trapping (1), we find that 0.4 and 2.8% of the $a < 34$ AU population is, at any time, in co-orbital motion with Uranus and Neptune, respectively [with less than a factor of 2 variation (1)]. This 3.2% fraction is much larger than the $\approx 0.1\%$ of near-Earth asteroids temporarily trapped in Earth and Venus co-orbital motion (14, 15), presumably due to the fractionally larger co-orbital regions of the giant planets. We find that the simulated Uranian and Neptunian co-orbitals consisted of, respectively, 64 and 54% in horseshoe orbits, 10 and 10% quasi-satellites (1), and 26 and 36% Trojans, equally distributed between the L4 and L5 clouds. The duration of Uranian co-orbital captures in our simulation had mean, median, and maximum values of 108 ky, 56 ky, and 2.6 My, respectively, and 78 ky, 46 ky, and 18.2 My, respectively, for Neptune.

To explore the strength of observational biases, we used the CFEPS Survey Simulator (20), expanded to include our additional coverage. The survey simulator applies observational biases to the population model (Fig. 3, top) from the dynamical simulation (1) to simulate what a survey would observe (Fig. 3, bottom). The absolute H -magnitude distribution (a proxy for the size distribution) of objects is important when modeling flux-limited surveys. Our first attempt to use a single exponential distribution [$dN/dH \propto 10^{\alpha H}$ (1)] with $\alpha \approx 0.8$, as measured for $H_g < 9.0$ TNOs

(16, 21) and Neptunian Trojans (5), was rejected at a high level of confidence, predicting that small ($H_g > 11.0$) objects should account for 81% of the detections; our observations have no such objects. The H -mag distribution of Neptunian Trojans cannot continue as a single exponential (5) beyond $m_R \approx 23.5$ ($H_g \approx 9.3$, assuming $g - R = 0.5$ and a typical distance of 30 AU). The scattering objects also reject a single exponential and are better represented by a “divot” H -mag distribution (22), where the number density drops at $H_g \approx 9.0$ by a contrast factor (ratio of density just before and after the divot) of 6, then continues with a second shallower exponential with $\alpha \approx 0.5$. Although we lack the statistics to independently constrain a divot, simply adopting the above parameters provides better agreement between simulated and observed populations (Fig. 3), with small ($H_g > 11.0$) objects only providing 23% of the simulated detections.

Simulating our available calibrated fields, we find that 0.9% of the $a < 34$ AU detections should be Uranian co-orbitals and 2.2% should be Neptunian co-orbitals (Fig. 3). Thus, because of where we looked and the survey limits, detection of Uranian co-orbitals was enhanced compared to the intrinsic fraction (0.4%), whereas the Neptunian co-orbitals were slightly biased against (relative to 2.8%). Of the 247 objects with 6 AU $< a < 34$ AU currently in the MPC database, about six (including 2011 QF₉₉) have been identified as temporary co-orbitals of Uranus and Neptune (1), yielding $\sim 2.4\%$. This is within a factor of 2 of our 3.2% model prediction, although the unknown pointing history and survey depths make detailed modeling impossible. Because our simulations show that no large (not even factor of 2) biases exist toward or against detecting co-orbitals, the MPC co-orbital fraction may be close (within a factor of a few) to the intrinsic fraction. Thus, 2011 QF₉₉ is a Uranian Trojan that is part of a roughly constant population of transient co-orbitals, temporarily (although sometimes for millions of years) trapped by the giant planets, similar to those seen for the terrestrial planets (14, 15, 23).

Fig. 3. Results of our survey simulations. (Top) 10^6 objects drawn from the model population of scattering objects (tiny cyan dots), Uranian co-orbitals (red), and Neptunian co-orbitals (blue), used as the intrinsic population in our survey simulation. The populations have the relative fractions from the model, but the co-orbitals have larger symbols to enhance visibility. **(Bottom)** Objects detected by our survey simulator, using the same color scheme. The large black squares are the four real $a < 34$ AU detections from our calibrated work, 2011 QF₉₉ being the upper-left one. Black curves correspond to apparent magnitudes $m_g = 24.25$, 25.25, and 26.25, from bottom to top, which are roughly the survey limits of CFEPS (16), our new observations, and a deep survey for Neptunian Trojans (5), respectively. The effect of the divot H -mag distribution can be seen around $H_g = 9.0$



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Supplementary Materials

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Paleofluvial Mega-Canyon Beneath the Central Greenland Ice Sheet

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Subglacial topography plays an important role in modulating the distribution and flow of basal water. Where topography predates ice sheet inception, it can also reveal insights into former tectonic and geomorphological processes. Although such associations are known in Antarctica, little consideration has been given to them in Greenland, partly because much of the ice sheet bed is thought to be relatively flat and smooth. Here, we present evidence from ice-penetrating radar data for a 750-km-long subglacial canyon in northern Greenland that is likely to have influenced basal water flow from the ice sheet interior to the margin. We suggest that the mega-canyon predates ice sheet inception and will have influenced basal hydrology in Greenland over past glacial cycles.

Greenland is an ancient craton dominated by crystalline rocks of the Precambrian shield, composed largely of Archaean [3100 to 2600 million years (My) before the present (B.P.)] and Proterozoic (2000 to 1750 My B.P.) formations, with more limited sedimentary deposits along parts of the continental margins (1). These rocks are resistant to glacial erosion compared with the sedimentary deposits that are thought to underlie substantial sectors of Antarctica (2, 3). Although there is evidence for some glaciation dating back to 38 My B.P. (4), the island has only been extensively ice-covered for no more than ~3.5 My (5), as compared with the Antarctic ice sheet that first grew at ~34 My B.P., and has existed persistently since ~14 My B.P. There are, therefore, important geologic and glacio-morphological differences between the subglacial environments of the two ice sheets.

Ice-penetrating radar (IPR) is capable of imaging the ice-sheet base and, if collected as a series of transects, can reveal the landscape beneath the ice. In Greenland, such data have been acquired on numerous occasions over the past 40 years (6, 7). Using these data in combination with ice-surface topography measurements, a quasilinear feature was previously identified in northern Greenland from a combination of ice-surface

topography and IPR data, but with limited knowledge on its extent and configuration (8). By inspecting IPR-derived basal topography from these and more recent data sources, we have discovered this bed feature to be a major subglacial canyon that extends from almost as far south as Summit in central Greenland to the northern margin of the ice sheet, terminating in the fjord that drains Petermann Gletscher (Fig. 1). The extent of the canyon is at least ~750 km, but its southern limit may continue further than we are able to identify because the density of IPR tracks below 74.3° N is limited. However, two tracks at 73.8° N, 42° W cross an over-deepening that appears likely to be the southern extension of the canyon. There is no obvious evidence of a geological boundary in this part of Greenland (9). The southern limit of the canyon, however, does lie close to a minimum in the free-air gravity anomaly and a maximum in the terrain-correlated gravity anomaly, which indicates crust that is isostatically thin and subject to compressive inflow of crustal material (9), which could form an explanation for its genesis.

The IPR data indicate that the canyon has a depth and width of up to ~800 m and ~10 km, respectively, toward the coast and a slightly reduced width and substantially shallower depth of ~200 m further south (Fig. 2 and figs. S1 and S2). The valley width-to-height ratio (used as a measure of river channel morphology and formation) for the profile in Fig. 2C is 30, which is indicative of a broad, shallow channel, subject to limited stream erosion (10, 11). The ratio for the profile in Fig. 2A is ~0.5, suggesting linear stream in-

cision was important in the development of the channel at this location, with no evidence of glacial erosion being the dominant process (12). Indeed, none of the profiles are typical of glacially eroded valleys (fig. S2). The canyon follows a

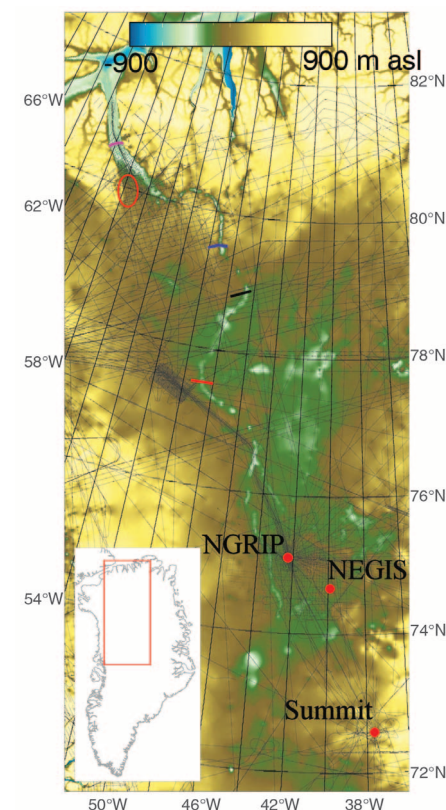


Fig. 1. Bed elevation [between -900 and 900 m above sea level (asl)] for northern Greenland. The area plotted is indicated by the red box in the inset. Airborne ice penetrating radar flight lines are shown in gray, and the three bed profiles plotted in Fig. 2 are shown by the solid blue (Fig. 2A), black (Fig. 2B), and red (Fig. 2C) lines. The mauve line shows the approximate location of the grounding line on Petermann Gletscher. Other places discussed in the text are also labeled: NGRIP, the onset area of the North East Greenland Ice Stream (NEGIS), and the camp near the highest point of the ice sheet, Summit. The red ellipse marks the approximate location of a marked minimum in crustal thickness, and hence maximum in geothermal heat flux, identified from gravity data (9).

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meandering path more typical of a large river system. The dimensions of the Greenland canyon are comparable with parts of the Grand Canyon in the United States in terms of width and length and, in its deepest region, are about half of the Grand Canyon's depth.

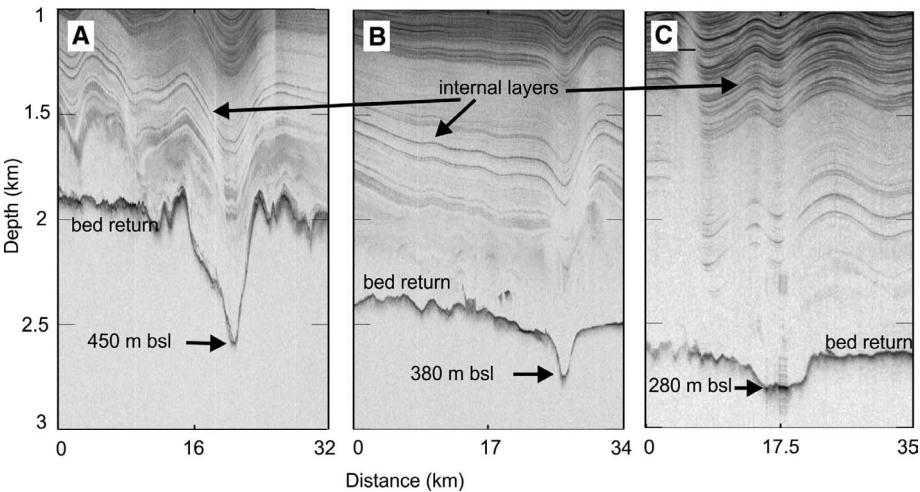


Fig. 2. Ice-penetrating radargram profiles across the canyon at three locations. The locations are indicated in Fig. 1 by (A) the solid blue line, (B) the black line, and (C) the red line. There is an exaggeration in the vertical by a factor of 26. The depth of the canyon in meters below sea level is indicated in each profile alongside internal reflections within the ice and the bed return. North is roughly into the page.

To assess the canyon's influence on the flow of subglacial water, and the extent to which it was a mega-channel for surface water, we calculated the hydraulic potential for both the present-day, ice-free, and last glacial maximum (LGM) configurations of the Greenland ice sheet (Fig. 3), accounting where necessary for isostatically compensated ice-free bedrock. We used the methods described in Shreve (1972) (13), which have been commonly applied to determine subglacial hydrological pathways (14). Because of its size, the canyon provides a hydraulic pathway in all cases tested, suggesting that it has influenced basal water flow in northern Greenland since ice sheet inception and, indeed, before this. For the ice-free (preglacial) state (Fig. 3A and fig. S3B), water is predicted to flow south-to-north along the majority of the canyon. The isostatically compensated channel gradient, without the ice sheet load, is 0.3 m km^{-1} , which is similar to the southern 500 km of the Colorado River and around three times larger than the average for the Mississippi River. Regions where water diverts away from the canyon (such as at 79.3° N , 48° W) are coincident with limited IPR data coverage (Fig. 1), implying poor resolution of the bed topography in such places. Hence, we believe water will likely have routed

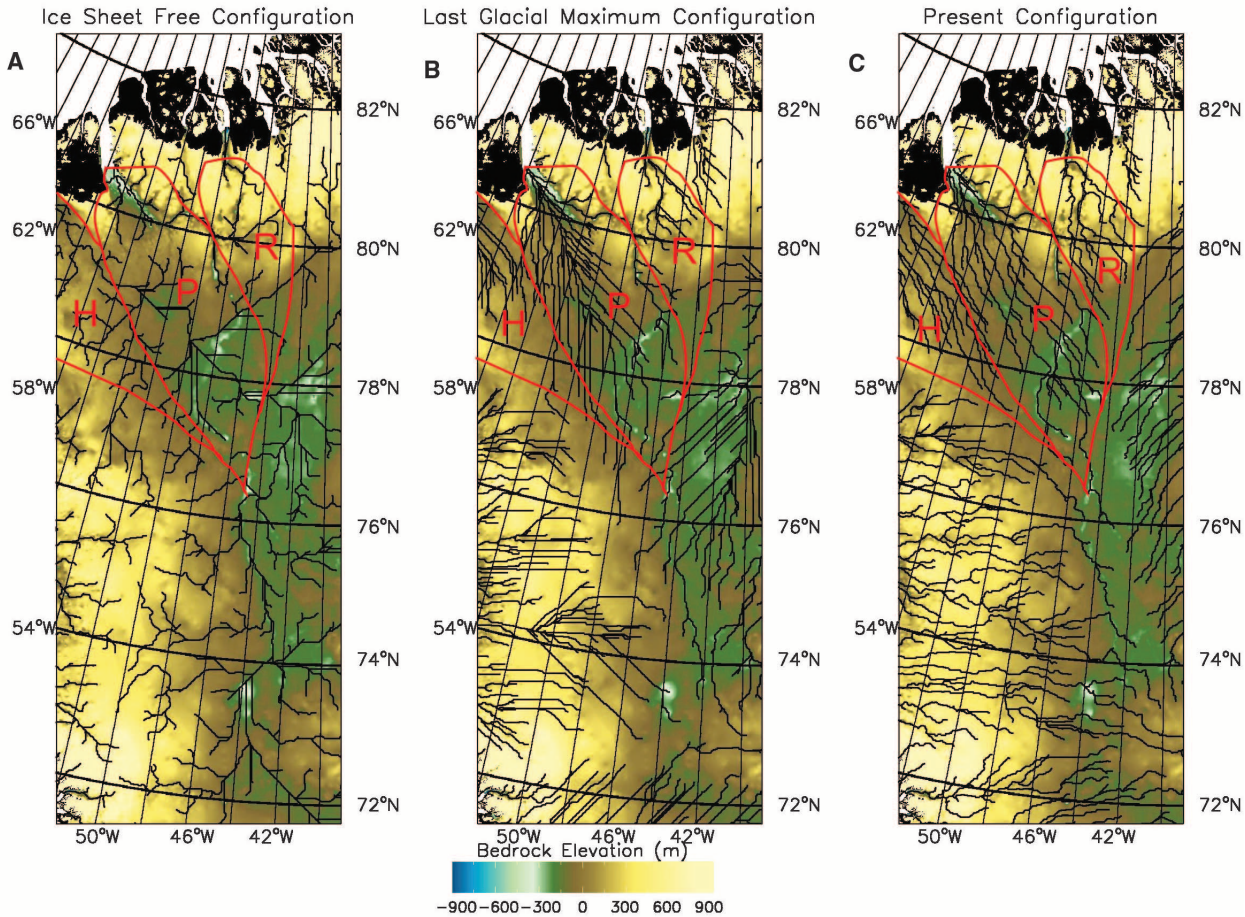


Fig. 3. Subaerial and subglacial hydraulic potential flowpaths. (A) An isostatically compensated bed without an ice sheet. (B) An ice sheet configuration at LGM. (C) The present-day ice sheet and bed configuration. H, Helheim; P, Petermann; R, Ryder. The bed elevations shown are all for present day. The isostatically compensated bed elevation is illustrated in fig. S3B.

continually along this feature before extensive ice sheet formation ~3.5 My B.P. (5).

In full glacial conditions [based on LGM (supplementary materials)], the flow of water is influenced by the ice overburden pressure, which forces water out of the canyon in parts of the upper Petermann catchment and along the direction of ice flow (Fig. 3B). Nonetheless, the canyon remains a conduit for the flow of basal water toward the coast even under LGM conditions, especially northward of ~79° N. For the present ice-sheet configuration, which is small and short-lived compared with its expanded full glacial state, steeper ice surface slopes (relative to LGM) force more water from the canyon to the west at certain locations (Fig. 3C and fig. S3A). In all cases, above ~76° N and within the entire length of the Petermann catchment, the canyon exerts a control on basal water flow. For ~200 km, it provides an uninterrupted hydraulic pathway (Fig. 3 and fig. S3A) that ends at the terminus of Petermann Gletscher. However, although extensive parts of northern Greenland have been identified as having a wet bed (fig. S4) (15), water currently may not be ubiquitous throughout its length. Under full glacial conditions, the ice sheet was larger and thicker than at present day (4), and therefore, a greater proportion of the bed was likely melting during the longer lasting LGM ice sheet configuration.

The lack of substantial subglacial lakes in the main Greenland ice sheet may be partly explained by generally steeper ice surface slopes as compared with those of Antarctica, but subglacial water must be evacuated by some means or it will pool. The subglacial canyon provides an efficient pathway for this water routing over an otherwise relatively flat bed (Fig. 3C). Given that water beneath former ice sheets in Greenland was likely to have been influenced by the canyon, it seems probable that conditions have never been well suited to substantial long-term basal water storage in this sector of Greenland.

The floating ice shelf in front of Petermann Gletscher has been found to possess sub-ice-

shelf channels 1 to 2 km wide and 200 to 400 m deep incised upward into the ice (16). These channels are associated with high basal melt rates, exceeding 30 m year⁻¹. Their existence has been attributed entirely to the action of the ocean (16). We believe the efficient routing of basal water via the canyon to the northern ice sheet margin is also important in explaining these features. Analysis of IPR echo-strength data has identified extensive regions of the northern half of the Greenland ice sheet that have water at the bed (15). Subglacial melt rates in the vicinity of the North Greenland Ice Core Project (NGRIP) drill site have been estimated to exceed 1 cm year⁻¹ (17) and are as high as 15 cm year⁻¹ near the onset of the Northeast Greenland Ice Stream (18). A study examining crustal thickness and geothermal heat flow in Greenland found a minimum in the former (and hence, a maximum in the latter) close to the northern limit of the canyon (Fig. 1) (9). Thus, substantial volumes of subglacial meltwater are generated at the bed, and additional surface meltwater may also penetrate to the bed near the coast (19). An effective means by which this basal water is routed to the ice-sheet margin must exist, else subglacial lakes would form. Basal water generated in the Petermann catchment is highly likely to be routed through the Canyon to the ice sheet terminus (Fig. 3C). Thus, basal water is likely to be delivered at a point source to the ice-shelf cavity, which is likely to influence the local subshelf water circulation (20). Similar sub-shelf channels have been identified underneath Pine Island glacier and elsewhere in Antarctica (21), and we suggest that well-organized subglacial meltwater flow is likely to play a similar role in the development of these features.

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Supplementary Materials

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Social Learning of Migratory Performance

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Successful bird migration can depend on individual learning, social learning, and innate navigation programs. Using 8 years of data on migrating whooping cranes, we were able to partition genetic and socially learned aspects of migration. Specifically, we analyzed data from a reintroduced population wherein all birds were captive bred and artificially trained by ultralight aircraft on their first lifetime migration. For subsequent migrations, in which birds fly individually or in groups but without ultralight escort, we found evidence of long-term social learning, but no effect of genetic relatedness on migratory performance. Social learning from older birds reduced deviations from a straight-line path, with 7 years of experience yielding a 38% improvement in migratory accuracy.

Mechanisms underlying the complex phenomenon of animal migration have been particularly well studied in birds (1–5).

In some taxa, individuals may migrate alone, unaided by conspecifics and relying instead mostly on endogenous, genetically inherited navigation

programs (6). In other species, innate programs alone are not sufficient, and experiential learning is critical to successful navigation, as adult animals often have markedly better navigational capabilities than juveniles (7–9). Information transfer from more experienced individuals to inexperienced ones can be essential to navigational success,

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especially for species that travel in groups (8–10). Current hypotheses, richly supported by theoretical studies (11–13), posit that social learning, coupled with interindividual coordination of movements, is essential to successful migration and the maintenance of group structure. Despite these advances, disentangling the contributions of experience and social learning (mediated through cultural transmission of knowledge) versus innate navigation programs (inherited genetically) to migratory performance represents a key challenge in understanding animal behavior (2).

To address that challenge, we used databases emerging from long-term investment in the conservation of whooping cranes (*Grus americana*). Formerly widespread in North America, whooping cranes are now endangered and restricted to a relictual wild population, migrating between northwestern Canada and the Texas coast, and birds that originate from reintroduction efforts. The most successful reintroduction effort to date established the eastern migratory population (EMP). Most birds in the EMP migrate between a summering range centered on Necedah National Wildlife Refuge in Wisconsin and a wintering range in the area of Chassahowitzka National Wildlife Refuge in Florida (Fig. 1).

We used 2002–2009 relocation data for the EMP, which have two distinct features that allow us to partition genetic and socially learned aspects of migration. First, the EMP is wholly derived from a captive breeding program, and the pairwise genetic relatedness of all migrants is known from studbook pedigrees (14). Second, new captive-bred, naïve-to-migration birds were transferred each summer to Necedah National Wildlife Refuge and, during their first fall, were trained on their southbound migration route by human-piloted ultralight aircraft (15, 16). Thus, all birds were initially trained to follow the same migration route. Although another release method has been used in this population more recently, we included only ultralight-trained birds in our study. After this first training flight, the cranes migrate freely, flying in groups with other cranes but without ultralight aircraft on all subsequent north- and southbound migrations. Because of their endangered status, the stepwise progression of each bird's route on each migration is intensively monitored, yielding both detailed spatial information and comprehensive information on group composition on each trip.

The necessity for ultralight training suggests that successful migration in whooping cranes depends on both social learning and innate programs. As in storks (17), southward autumn migrations by naïve-to-migration, captive-reared juveniles flying in the absence of experienced individuals would be unlikely to lead to a successful journey, suggesting that cultural transmission of information is important (15). Innate programs influence initiation of migration in that ultralight-trained birds can initiate the northbound spring migration independently of experienced birds (or ultralight aircraft) (15).

To disentangle the effects of social learning and innate navigation programs on migratory performance, we extracted data on four predictor variables from the crane databases: (i) the age of all individuals on each flight (which we hypothesize as a measure of experiential learning), (ii) the age of the oldest individual(s) in a migrating group of cranes (which we hypothesize provides an upper limit on experiential learning within each group, as one or more individuals may be the oldest in a group), (iii) the group size as a measure for potential group navigation, and (iv) genetic relatedness (which captures interindividual nonindependence in the birds' innate navigation programs). We used deviations from a straight-line path between summer and winter ranges on the migratory route of individual birds as a proxy for migratory performance and built a hierarchical linear mixed model to examine how much of those deviations at each observed location on the migratory route could be explained by individual age, age of the oldest individual(s) in a migratory social group, group size, and genetic relatedness on both individual and group levels (18). In addition, our model included the effects of sex and season (18).

Social learning facilitated long-term increases in the accuracy of migration. The age of the oldest individual(s) in a group improved migratory performance by ~5.5% per year of age (Fig. 2), decreasing the average deviation from a straight-line path by ~4.2 km per year of age for each relocation event [posterior mode: -4.2 km, 95% highest posterior density interval (HPDI): -1.1 to -7.2 km]. Flight groups in which the oldest individual(s) had a migratory age of 1 year were predicted to deviate ~76.1 km from the straight-line path per relocation, whereas in groups in which the oldest individual(s) was 8 years old, the predicted deviation was only 46.8 km. Thus, 7 years of experience translated into a 38% improvement in migratory performance (Fig. 2). Overall, autumn locations were predicted to deviate 36.3 km more from the straight-line paths than did spring locations (95% HPDI: -21.0 to -54.3 km) (Fig. 2). We found no significant effects of sex (posterior mode: 2.4 km, 95% HPDI: -2.9 to 8.8 km), individual migratory age (as opposed to age of the oldest bird in a group) (posterior mode: -1.4 km, 95% HPDI: -3.8 to 1.2 km), or group size (posterior mode: -0.3 km, 95% HPDI: -4.8 to 5.8 km). The lack of an effect of group

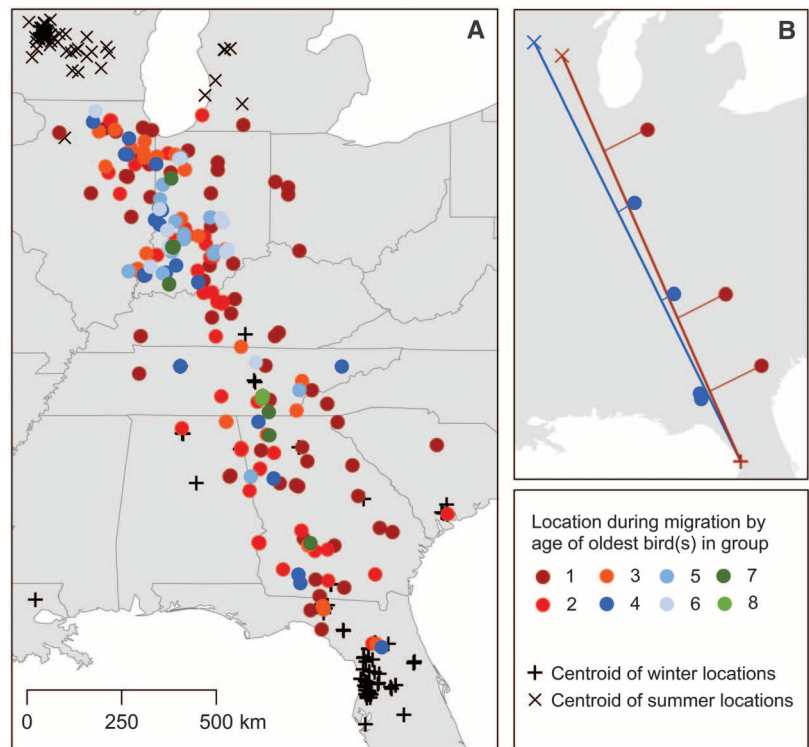


Fig. 1. Whooping crane location data. (A) Migration map for the EMP of whooping cranes (2002–2009). We identified each bird's summer and winter ranges in each year using the mean coordinates of all locations for that individual during summer and winter times when birds are not migratory. We then identified the straight-line path for each migration event linking consecutive summer and winter (or winter and summer) ranges for each bird. We calculated the deviation of each migratory relocation from the straight-line path and used this as a simple proxy for migratory performance. Variation in data availability over the 8 years of the study precluded application of more complex measures of deviation, such as those based on full trajectories that might take into account heterogeneity in wind strength and direction, topography, and the availability of suitable stopover sites. (B) Typical migratory pattern for two 1-year-old individuals migrating in spring 2005 traveling without (red) and with (blue) older birds.

size is interesting, as theory suggests that larger group sizes may aid navigation (13). In addition, a fixed effect for mean group genetic variance [i.e., the mean breeding value (19) potentially predicting better migratory accuracy] was indistinguishable from zero (standardized posterior mode: -0.03 km, 95% HPDI: -10.7 to 24.9 km) (Fig. 2). This finding implies that closely related birds did not migrate more similarly to each other (either increased or decreased accuracy) than did less related birds.

On average, 1 year olds that traveled with older birds deviated by 63.9 km from the straight-line paths, which was 34% less than for 1 year olds that traveled in same-age groups (mean deviation: 97.1 km) (Fig. 3). Accounting for other sources of variation, the modeled difference was 44.7 km (95% HDPI: 6.6 to 85.7 km). Groups of 1-year-old birds migrating without older birds were particularly prone to large deviations from the straight-line route. Fully 25% of locations of 1 year olds flying without older birds exceeded 150 km of deviation, the largest deviation observed for mixed-age groups (Fig. 3).

Previous research has contributed to overall understanding of the role of experience, social transmission of knowledge, and innate programs in navigation and route-learning of birds. How-

ever, results must be drawn piecemeal from across diverse studies. For example, translocation experiments involving several bird species have demonstrated individual learning (9), social learning (20), and innate programs (21) in isolation. The crane analyses reported here provide an integrated, multiyear portrait of these critical issues within a single species.

We show that learning of migration routes by whooping cranes takes place over many years and that social transmission of knowledge by experienced older birds yields progressive improvements in migratory performance of younger birds. In cranes, social learning may contribute to improved navigation through spatial memory of landscape features. Tracking studies on the relictual wild population of whooping cranes suggest that memory of landmarks across small scales and long-distance responses to large-scale topography may aid navigation (22, 23). Experience may also manifest as improved coping with weather patterns such as wind drift, as has been shown for raptors (24). More than 75% of our relocations were to the east of the straight-line migratory path, which accords with the predominantly westerly winds in the region (fig. S1). The absence of experienced adults may lead to failed or misdirected migration (8, 17). In our study, we found that

younger birds lack accuracy only when unaided by older birds (Fig. 3), suggesting the absence of intentional exploration. However, greater deviations in the fall migration may arise from explorations in search of alternative overwintering areas, because birds do not always return to the initially trained overwintering area (Fig. 1A).

In other bird species, the timing and direction of migration are strongly heritable (6). Even though we did not find a significant effect of genetic relatedness on migratory performance, other evidence indicates that innate programs must play a role in some aspects of crane migration. For example, the first independent northward migration can be initiated by flight groups that consist of only juvenile birds, demonstrating that some elements of migration knowledge need not be culturally transmitted in whooping cranes.

Beyond their contributions to understanding social learning of migration, our findings have important implications for conservation and reintroduction efforts of whooping cranes. If experience and learning accrue with time for crane reproduction, as demonstrated here for migration, additional experience may also improve successful reproduction in the wild, especially given the potential links between migratory performance and breeding performance (25). Because the average age of the whooping crane EMP is itself increasing, further improvements in migratory performance are expected. Previous studies have demonstrated that leadership plays an important role in bird homing navigation and that more experienced birds are more likely to become leaders (26); our results show that social learning enhances group navigation performance for long-distance migrants and that the benefits of experience accrue over many years.

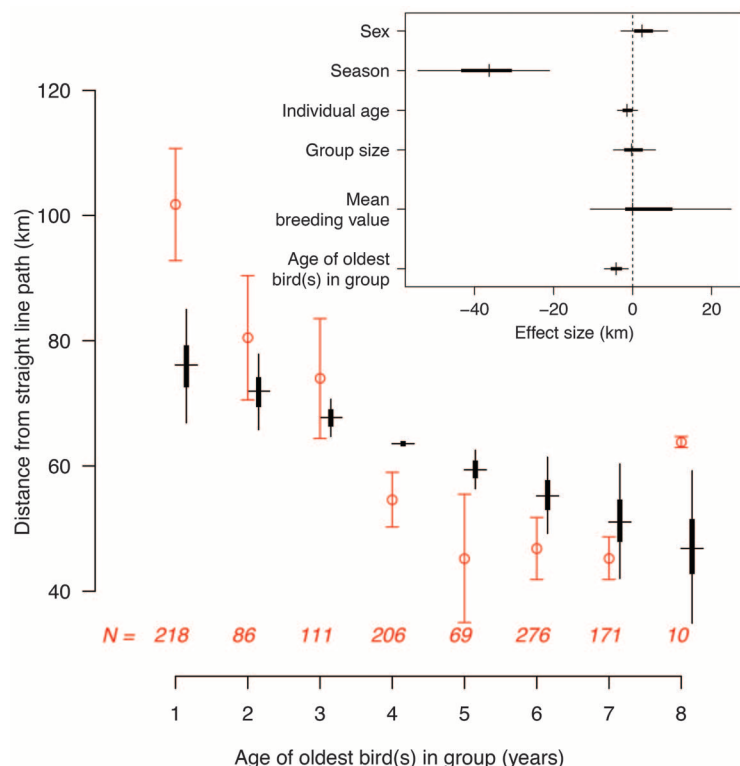


Fig. 2. Migratory performance of whooping cranes measured as deviation of locations from straight-line path versus age of oldest individual(s) in each flight group with more than one individual. Original data [red; means with 95% confidence intervals (CIs)] and model predictions (black; posterior modes, quartiles, and 95% HPDI) accounting for variation in several other factors, including migratory season, bird sex, and the birds' genetic relatedness, are shown. Sample sizes (N) are relocation events. (Inset) Posterior distributions of overall model terms. Mean additive genetic variance refers to the group effect of genetic variance [i.e., the mean breeding value (18)].

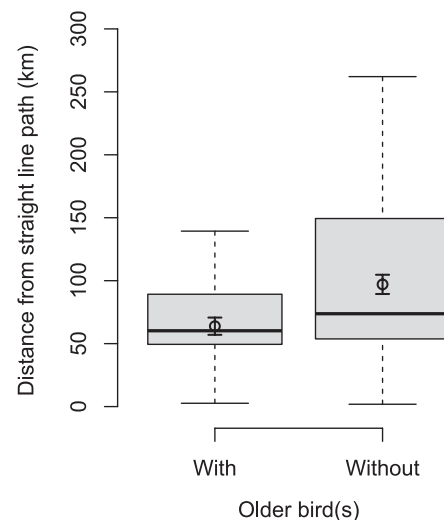


Fig. 3. Distance from straight-line path for locations of 1-year-old birds that migrated with older bird(s) compared with 1-year-old birds that migrated in groups without older bird(s). Box plots providing minimum, maximum, medians, and upper and lower quartiles are shown in gray. Means and 95% CIs are shown inside the box plots.

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Supplementary Materials

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Nuclear Wave1 Is Required for Reprogramming Transcription in Oocytes and for Normal Development

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Eggs and oocytes have a remarkable ability to induce transcription of sperm after normal fertilization and in somatic nuclei after somatic cell nuclear transfer. This ability of eggs and oocytes is essential for normal development. Nuclear actin and actin-binding proteins have been shown to contribute to transcription, although their mode of action is elusive. Here, we find that *Xenopus* Wave1, previously characterized as a protein involved in actin cytoskeleton organization, is present in the oocyte nucleus and is required for efficient transcriptional reprogramming. Moreover, Wave1 knockdown in embryos results in abnormal development and defective *hox* gene activation. Nuclear Wave1 binds by its WHD domain to active transcription components, and this binding contributes to the action of RNA polymerase II. We identify Wave1 as a maternal reprogramming factor that also has a necessary role in gene activation in development.

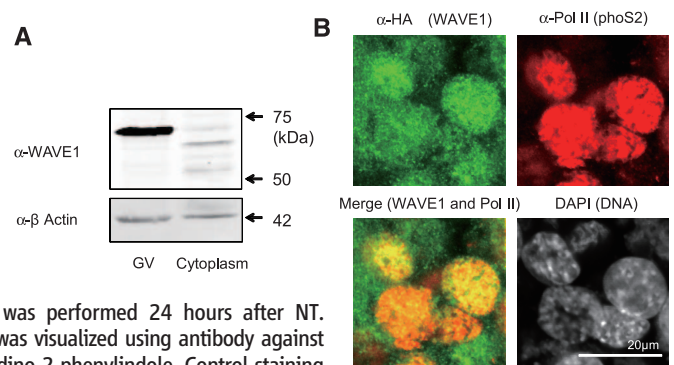
Eggs and oocytes efficiently reprogram transplanted somatic nuclei to an embryonic state (1, 2). This reprogramming ability of eggs and oocytes toward somatic nuclei is believed to relate to their natural activity to activate sperm nuclei at fertilization. Reprogramming factors are synthesized and accumulated during egg formation and are especially enriched in the amphibian oocyte nucleus, named the germinal vesicle (GV) (1). GVs also contain necessary factors for embryonic development (3, 4). It is unclear what

kinds of GV factors are required for reprogramming and for normal development, and how they contribute to these fundamental processes. To iden-

tify such a maternal factor, we have developed a nuclear transfer assay; hundreds of mammalian somatic cell nuclei are injected into the GV of *Xenopus* oocytes, and these nuclei undergo not only continuous transcription of active genes but also transcriptional reactivation of somatically silenced embryonic genes within 2 days (1). This system thus provides a unique opportunity to identify maternal factors responsible for reprogramming the transcription of somatic nuclei.

Previously, we found an important role of oocyte nuclear actin in transcriptional reprogramming (5). Actin dynamics are regulated by actin-binding proteins (ABPs) (6). Increasing evidence suggests that nuclear ABPs play crucial roles in transcriptional activation (7–9). Therefore, we tested the roles of nuclear ABPs in reprogramming and development. The effect of overexpressing ABPs in recipient *Xenopus* oocytes on transcriptional reprogramming of *Pou5f1* (*Oct4*) was examined by reverse transcription quantitative polymerase chain reaction (RT-QPCR) (fig. S1A) (10). Overexpression of two ABPs Tocal1 (5) and Rac1 significantly enhanced *Oct4* transcription from transplanted mouse C2C12 myoblast cell nuclei ($P < 0.01$) (fig.

Fig. 1. Wave1 is present in the *Xenopus* oocyte nuclei and transplanted mouse nuclei. (A) Western blot analysis revealed that Wave1 is accumulated in the GV of the *Xenopus* oocyte. (B) Mouse C2C12 nuclei were injected into the GV overexpressing HA-NLS-Wave1 (fig. S1A). Im-



munofluorescence analysis was performed 24 hours after NT. HA-NLS-Wave1 localization was visualized using antibody against HA (αHA). DAPI, 4',6'-diamidino-2-phenylindole. Control staining is shown in fig. S3.

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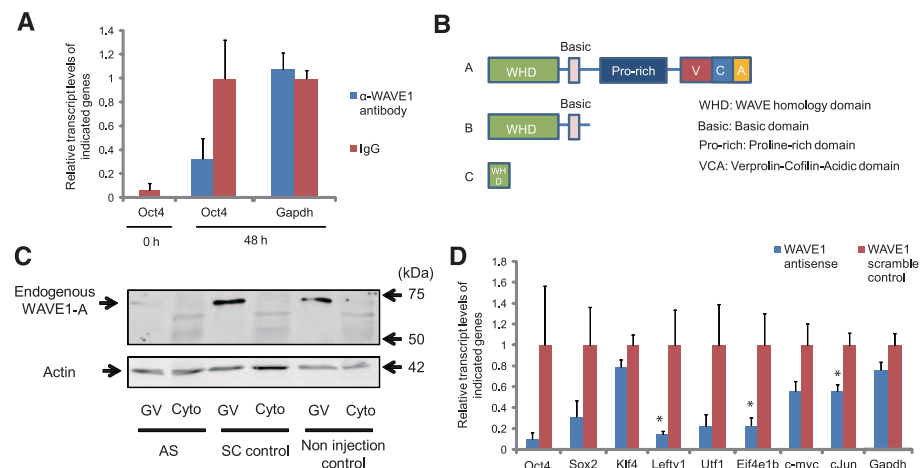


Fig. 2. Nuclear Wave1 is required for transcriptional reprogramming in oocytes. All error bars represent SEM. $*P < 0.05$. (A) Injection of antibody against Wave1 into GV inhibits the transcriptional activation of *Oct4* in NT oocytes ($n = 12$). Immunoglobulin G was injected as control ($n = 13$). Relative fold increases of gene transcripts to control NT were measured by QPCR. (B) Three transcript variants expressed in *Xenopus* oocytes were identified (A, B, and C), and each protein domain is represented by different colors. (C) Knockdown of endogenous Wave1 proteins in the GV by antisense oligonucleotide injection (AS) as checked by Western blot. SC control means scrambled oligonucleotides-injected control. Cyto represents the oocyte cytoplasm. (D) Knockdown of Wave1 by AS inhibits transcriptional activation of many embryonic genes in NT oocytes (blue bars). $n = 6$.

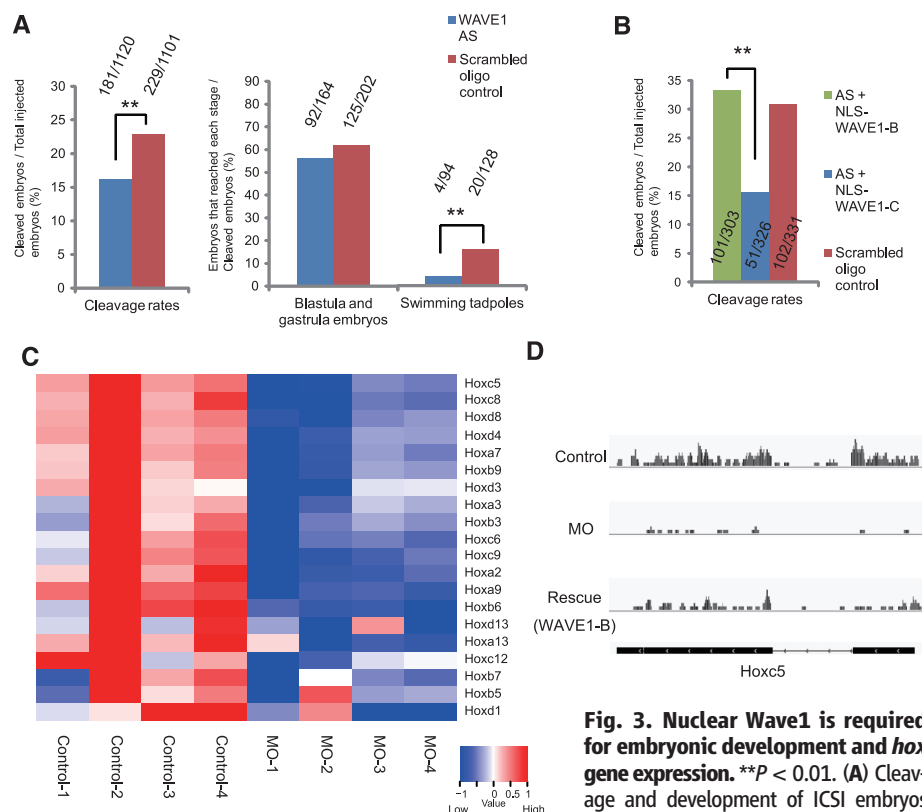


Fig. 3. Nuclear Wave1 is required for embryonic development and *hox* gene expression. $**P < 0.01$. (A) Cleavage and development of ICSI embryos are significantly impaired by Wave1 knockdown with AS. Actual numbers of embryos treated and developed are indicated above the corresponding graphs. (B) Abnormal cleavage caused by Wave1 knockdown is rescued by overexpression of NLS-Wave1-B, but not by NLS-Wave1-C. (C) Heat-map analysis shows lower expression of *hox* genes in Wave1-MO embryos (MO1-4) than control embryos. (High expression in red and low expression in blue.) (D) RNA-seq analysis shows that down-regulated *hoxc5* expression by Wave1 MO injection is partially rescued by overexpressing NLS-Wave1-B. Black boxes in *hoxc5* gene represent exons.

down with AS. Actual numbers of embryos treated and developed are indicated above the corresponding graphs. (B) Abnormal cleavage caused by Wave1 knockdown is rescued by overexpression of NLS-Wave1-B, but not by NLS-Wave1-C. (C) Heat-map analysis shows lower expression of *hox* genes in Wave1-MO embryos (MO1-4) than control embryos. (High expression in red and low expression in blue.) (D) RNA-seq analysis shows that down-regulated *hoxc5* expression by Wave1 MO injection is partially rescued by overexpressing NLS-Wave1-B. Black boxes in *hoxc5* gene represent exons.

S1B). Downstream target proteins of Tocl and RAC1 are Wiskott-Aldrich syndrome protein (WASP) and WASP family verprolin-homologous protein (WAVE) (*11–13*). WASP is involved in RNA polymerase II (Pol II)-mediated transcription and in transcriptional activation during T cell differentiation (7, 8). However, N-wasp, the ubiquitous form of Wasp, is unlikely to be responsible for transcriptional reprogramming in *Xenopus* oocytes (supplementary text and fig. S1, C to E). Because the positive roles of N-WASP in transcription have been shown in cultured cells (8), oocytes may use different ABPs from somatic cells to regulate their productive transcription.

We tested the involvement of Wave in transcriptional reprogramming. WAVE plays a cytoplasmic role in actin reorganization as a downstream target of RAC (*13*). Rac1 protein is present in GV, including in transplanted nuclei (fig. S2A). To our surprise, Wave1, one isoform of Wave enriched in brain, is also accumulated in the oocyte GV (Fig. 1A). We therefore focused on Wave1 as a candidate reprogramming factor. Little WAVE1 is detected in C2C12 nuclei before nuclear transfer (NT) (green color, fig. S2B). After NT, the accumulation of Wave1 was observed, especially in some somatic nuclei that showed extensive swelling (white arrow, fig. S2B), known to correlate with high transcriptional activity (*14*). Accordingly, localization of Wave1 and active RNA Pol II phosphorylated at serine 2 was examined. *Xenopus laevis* Wave1 tagged with a nuclear localization signal (NLS) and hemagglutinin (HA) (HA-NLS-Wave1) was expressed in GV. A NLS was added to target Wave1 to the nucleus in order to focus on the nuclear role of Wave1. HA-NLS-Wave1 signals overlapped with active Pol II in transplanted nuclei (yellow color in the merged photo, Fig. 1B). Time course changes of HA-NLS-Wave1 and active Pol II in NT oocytes are shown in fig. S3. These results suggest that Wave1 is present in actively transcribing nuclei.

Subsequently, the importance of nuclear Wave1 in reprogramming embryonic genes in *Xenopus* NT oocytes was examined. We injected an antibody against Wave1 along with C2C12 murine myoblast nuclei to GV. Wave1 antibodies inhibited *Oct4* activation (Fig. 2A). We then asked whether overexpressing Wave1 enhances transcriptional reprogramming in oocytes. For this experiment, we cloned three transcript variants of *wave1* (*wave1-A*, *-B* or *-C*) from *Xenopus* oocyte cDNAs (Fig. 2B and table S1), although the variant C was rarely detected. All three transcripts were expressed in GV by mRNA injection (fig. S4A). Overexpression of Wave1-A and Wave1-B enhanced activation of embryonic genes (fig. S4B). Notably, Wave1-B significantly enhanced transcription from many of the genes examined, including housekeeping genes. In addition, we specifically knocked down oocyte Wave1 by antisense oligonucleotide (AS) injections (fig. S5A). ASs against *wave1* match sequences of both *wave1-A* and *wave1-B*. This method enabled us to stably decrease maternal *wave1* mRNAs (fig. S5B) and

to knock down Wave1 proteins (Fig. 2C). After injection of C2C12 nuclei into these Wave1-knocked down oocytes, activation of many embryonic genes was significantly impaired (Fig. 2D). We performed rescue experiments as depicted in fig. S5C using Wave1-B. Wave1-B expression successfully rescued activation of embryonic and oocyte-specific genes in oocytes ($P < 0.05$) (fig. S4C). Finally, we injected *Xenopus* sperm or 250 to 300 nuclei from XL177, a *Xenopus* cell line derived from tadpole epithelium, into oocytes having Wave1 knocked down to ask if Wave1 is important for transcriptional reprogramming in intraspecies NT oocytes. Several embryonic genes that were identified as candidate target genes of Wave1 by RNA-sequencing (RNA-seq) (described below) were activated after NT (fig. S6A). Transcription from such genes was significantly inhibited by Wave1 knockdown (fig. S6B). These results indicate that nuclear Wave1 is necessary for efficient transcriptional reprogramming in *Xenopus* oocytes.

We next asked whether nuclear Wave1 enhances transcriptional reprogramming in the induced pluripotent stem (iPS) cell system. NLS-Wave was transiently expressed at various time points during mouse iPS cell production using the doxycycline-inducible system (15). Wave1 expression at day 6 or 9 enhanced activation of pluripotency genes (fig. S7). This result suggests that *Xenopus* nuclear Wave1 can have a positive effect on the mammalian reprogramming system.

To test the idea that a GV factor important for reprogramming is also required for development, the role of Wave1 in embryogenesis was examined. We developed a system in which the knockdown of maternal Wave1 is achieved during early embryonic development (10). We injected ASs into oocytes, incubated for 1 or 2 days, and in vitro matured to metaphase II (MII) eggs (fig. S8A) (16). These Wave1 knockdown eggs were used for intracytoplasmic sperm injection (ICSI) (fig. S8A). We obtained a healthy frog from ICSI

embryos using in vitro matured eggs injected with control oligonucleotides (fig. S8B), which proved that this approach can support full-term development. Development of ICSI embryos was severely compromised by AS injection (Fig. 3A and fig. S8C). Moreover, the number of embryos that cleaved was decreased in AS-injected embryos (Fig. 3A), which suggested that Wave1 plays a role even before mid-blastula transition. These results agree with a previous report using bovine embryos (17). Abnormal cleavage was rescued by overexpressing NLS-Wave1-B (Fig. 3B). Wave1 inhibition in embryos was also accomplished by injection of morpholino oligonucleotides (MOs) into one-cell stage embryos (fig. S9A). This route toward knockdown is not effective at early cleavage stages, unlike AS injection to oocytes, and hence, defects in cleavage were not observed while development to the swimming tadpole is compromised (fig. S9B), in accord with the AS-knockdown results (Fig. 3A). These results indicate that nuclear Wave1 is required for normal embryonic development.

We then asked whether Wave1 is also involved in transcriptional activation in embryos. Transcriptional activation was analyzed by using Wave1 MO-injected embryos at the gastrula stage (stage12 to 12.5). RNA-seq analyses of Wave1 MO-injected embryos identified 964 misregulated transcripts among 23,560 total *Xenopus* transcripts (table S2) (420 down-regulated versus 544 up-regulated transcripts in Wave1 MO-injected embryos compared with control MO-injected embryos, false discovery rate < 0.05). Down-regulated genes identified by RNA-seq analyses were confirmed by RT-QPCR analyses (fig. S10). Pathway analysis of misregulated genes identified previously characterized functions of WAVE1, such as WASP family member and adenosine 3',5'-monophosphate (cAMP) response element-binding protein (table S3) (18), which suggested the specific inhibition of Wave1 functions. A number of other pathways are newly identified (table S3). Remarkably, many

hox genes were down-regulated in Wave1 MO-injected embryos (Fig. 3C). Down-regulation of *hox* genes transcription was partially rescued by expression of NLS-Wave1-B (Fig. 3D and fig. S11). In conclusion, nuclear Wave1 plays an important role in transcriptional activation in development. RNA-seq analyses also suggest that Wave1 seems to have specific downstream target genes rather than affecting global transcription in embryos.

We finally asked how nuclear Wave1 is involved in transcriptional activation. Results of our mass spectrometry experiments on Wave1-binding partners (supplementary text) prompted us to investigate whether nuclear Wave1 binds to the active transcription machinery. HA-NLS-Wave1-A, -B, and -C were expressed in embryos by mRNA injection, and extracts from these embryos were used for coimmunoprecipitation analysis. Active Pol II(phoS2) was bound to Wave1-A and -B, but not to Wave1-C (fig. S12A), in good agreement with transcriptional reprogramming experiments (fig. S4B). Wave1-A and -B were also coprecipitated with the C terminus of mixed-lineage leukemia (MLL) protein, which contains the SET domain and serves as a histone H3 lysine 4 (H3K4) methyltransferase. Although the protein band recognized by MLL antibody appeared lower than expected (19), we detected a specific band at the same size using human SET1 antibody (fig. S12B). We therefore concluded that the protein bound to Wave1 is a *Xenopus* SET domain-containing protein. These bindings were also observed when human WAVE1 was expressed (fig. S12C). This association was maintained even when the actin nucleation domain was removed from WAVE1 (13) (hWAVE1ΔVPH) (fig. S12C), which suggested that WAVE1 binding to the transcription apparatus is not through actin polymerization. We expressed different regions of the *Xenopus wave1* gene to identify a domain responsible for its binding to Pol II and the SET domain protein (Figs. 2B and 4A). The full Wave homology domain (WHD) was sufficient to mediate interaction with these

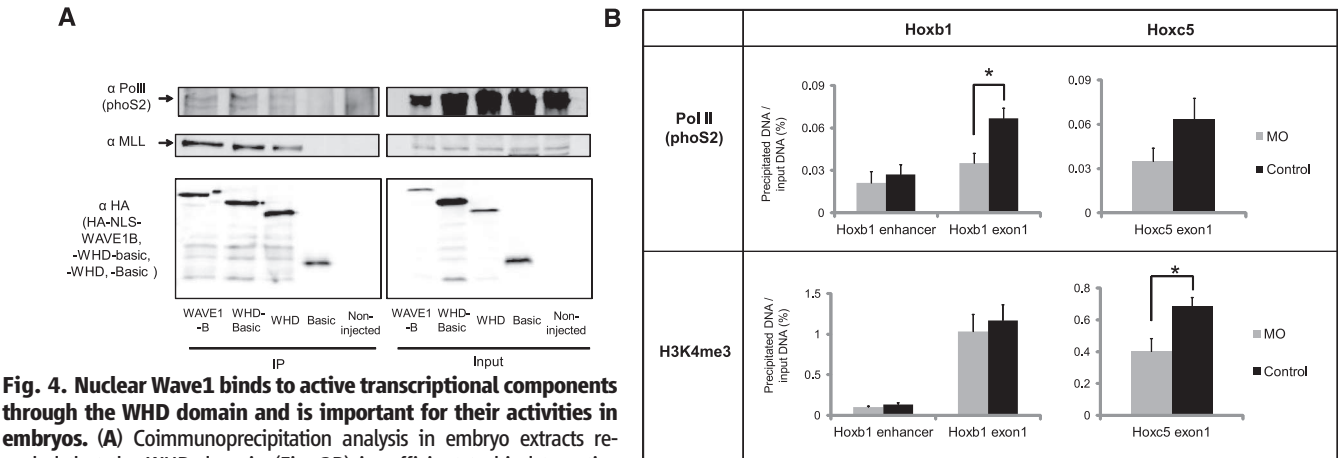


Fig. 4. Nuclear Wave1 binds to active transcriptional components through the WHD domain and is important for their activities in embryos. (A) Coimmunoprecipitation analysis in embryo extracts revealed that the WHD domain (Fig. 2B) is sufficient to bind to active Pol II and MLL. Five percent input was loaded. (B) Binding of elongating Pol II(phoS2) and H3K4me3 to *hoxb1* and *hoxc5* genes was examined by ChIP analyses. The levels of binding were compared in gastrula embryos with or

without Wave1 MO injection (MO and Control, respectively). Relative fold increases of precipitated DNA over input DNA were measured by QPCR. * $P < 0.05$. $n = 4$ (Pol II) and 8 (H3K4me3). Error bars represent SEM.

transcription regulators (Fig. 4A). We next checked the binding of nuclear Wave1 to *hox* genes by chromatin immunoprecipitation (ChIP) analysis. Overexpressed NLS-Wave1-B was specifically enriched on the *hox* genes enhancer regions (fig. S13, A and B). Finally, we examined levels of active Pol II and H3K4 trimethylation (H3K4me3) on *hox* genes in Wave1 knockdown embryos. ChIP analysis revealed that binding of elongating Pol II was down-regulated on *hoxb* and *hoxc* genes, and H3K4me3 was also reduced on *hoxc* genes (Fig. 4B and fig. S14). In conclusion, nuclear Wave1 associates with active transcription machineries through the WHD domain and modulates their activities on *hox* genes.

Our results demonstrate a role of Wave1 in transcription in oocytes and embryos and provide in vivo evidence that an actin-binding protein plays an important nuclear role in embryonic development. This nuclear function of Wave1 is due to its N terminus WHD domain (Fig. 4). It is noteworthy that the WHD domain mediates protein complex formation with kinase proteins for actin reorganization in the cytoplasm (18), which suggested that Wave1 can have differential binding partners between the cytoplasm and nucleus. This explains why Wave1 can have a different function in nuclei from that in the cytoplasm.

A long-standing question in developmental biology is which oocyte factors stored in the GV can contribute to embryonic development and reprogramming. Our study identifies a maternal reprogramming protein that is required for normal early development and shows its mechanism of action.

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Supplementary Materials

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References (20–36)

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Pou5f1 Transcription Factor Controls Zygotic Gene Activation In Vertebrates

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The development of multicellular animals is initially controlled by maternal gene products deposited in the oocyte. During the maternal-to-zygotic transition, transcription of zygotic genes commences, and developmental control starts to be regulated by zygotic gene products. In *Drosophila*, the transcription factor Zelda specifically binds to promoters of the earliest zygotic genes and primes them for activation. It is unknown whether a similar regulation exists in other animals. We found that zebrafish Pou5f1, a homolog of the mammalian pluripotency transcription factor Oct4, occupies SOX-POU binding sites before the onset of zygotic transcription and activates the earliest zygotic genes. Our data position Pou5f1 and SOX-POU sites at the center of the zygotic gene activation network of vertebrates and provide a link between zygotic gene activation and pluripotency control.

In early metazoan development, the zygotic genome is not immediately transcribed; instead, factors expressed during oogenesis from the maternal genome control development. The controlled synchronous onset of expression of the earliest large wave of zygotic transcripts is termed zygotic genome activation (ZGA). In vertebrates, ZGA is triggered by the change of nuclear-to-cytoplasmic ratio during early cleavage stages, as well as by other genome-level mech-

anisms (1). ZGA is one component of the maternal-to-zygotic transition (MZT) period, which also includes the gradual degradation of maternal transcripts (2). In *Drosophila*, the transcription factor Zelda (Zld) selectively primes the earliest zygotic genes for activation at MZT (3–8) and controls ZGA. Because no homologs of Zld have been reported outside the insect clade, it is unclear whether similar specific control of transcription onset exists in early vertebrate development.

In zebrafish, zygotic transcription starts during the midblastula transition (MBT), after the 10th cell division (9). Pou5f1 (also named Pou2 or Pou5f3; www.zfin.org), Nanog, and the functionally redundant SoxB1 group of transcription factors (Sox2, Sox3, Sox19a, and Sox19b) are ubiquitously present in the zebrafish egg and early embryo (10–13). Their homologs are con-

sidered core transcription factors of the mammalian embryonic stem (ES) cell state. In mammalian ES cells, the Pou5f1-Sox2 complex cooperatively assembles to target gene promoters containing bipartite SOX-POU binding sites (14–16). In zebrafish, loss of Pou5f1 function leads to severe disturbance of developmental progress immediately after MBT (17–20). Time-resolved expression analysis of MZspg mutants, devoid of both maternal and zygotic Pou5f1 function, revealed delays and desynchronization in expression of hundreds of genes (21), suggesting a role for Pou5f1 in temporal control of development. Here, we show that Pou5f1 in zebrafish selectively primes the earliest zygotic genes for activation, providing functionality similar to *Drosophila* Zelda (3–8).

To detect in vivo Pou5f1 and Sox2 chromatin binding events, we performed chromatin immunoprecipitation followed by parallel sequencing (ChIP-seq) and identified 7747 Pou5f1-bound regions at the pre-MBT 512-cell stage [2.75 hours postfertilization (hpf)], as well as 6670 Pou5f1-bound and 5924 Sox2-bound regions at the post-MBT stage (5 hpf; figs. S1 and S2 and table S1). Post- and pre-MBT Pou5f1 and Sox2 binding sites tended to colocalize (Fig. 1, A and D). To determine whether Pou5f1- or Sox2-bound genes correspond to targets of Pou5f1 and SoxB1 transcriptional regulation, we compared the list of genes bound by Pou5f1 or Sox2 within 20 kb upstream of their transcription start site (TSS) with lists of genes differentially expressed in embryos deficient for the respective transcription factors (13, 21). More than 100 genes activated by Pou5f1 and SoxB1 were directly linked to Pou5f1 and

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Sox2 binding regions, whereas no correlation with repressed genes was observed (fig. S3, A and B, table S2, and supplementary text).

We identified the top-scoring DNA binding motifs de novo from Pou5f1 and Sox2 ChIP-seq data. The calculated binding position weight matrices (PWMs) were bipartite, with SOX and POU binding halvesites. The post-MBT PWM closely resembled mammalian Oct4-Sox2 PWMs (Fig. 1, B and C, and table S3). However, the SOX halvesite in the PWM derived from pre-MBT Pou5f1 ChIP-seq was less pronounced (Fig. 1B and table S3), which suggests that Pou5f1 may use a broader range of transcriptional partners before MBT. Gene Ontology and expression annotations of genes located within 20 kb of pre- and post-MBT Pou5f1 and Sox2 ChIP-seq peaks were enriched for developmental genes, signaling pathway components, and factors with early regionalized expression (fig. S3C). Pou5f1 and Sox2 were bound to the regulatory regions of many orthologs of known targets of mammalian Pou5f1 and Sox2 in ES cells (14–16) including *pou5f1*, *nanog*, *sox2*, and *sox3* (Fig. 1D, fig. S4, fig. S5, A and B, table S4, and supplementary text).

Simultaneous and steeply increasing transcription of the earliest zygotic gene set is characteristic for ZGA (22, 23). To test whether Pou5f1 or SoxB1 may control zebrafish ZGA, we investigated whether post-MBT Pou5f1 and Sox2 sites are specifically enriched in the regulatory regions of the earliest-

expressed zygotic genes. To define the earliest zygotic gene group, we clustered our previously obtained expression data (21) into 30 groups defined by changes of expression during normal development: five 1-hour time windows (3 to 8 hpf), each further divided according to six criteria for relative change in expression (up-regulated by a factor of >5, 2 to 5, or <2; down-regulated by a factor of <2, 2 to 5, or >5; fig. S6A). We defined 162 genes that were up-regulated by a factor of >5 between 3 and 4 hpf as belonging to the “ZGA group” (Fig. 2A and table S5). We compared the fraction of genes close to Pou5f1 and Sox2 sites in each of the 30 above-defined expression groups. Pou5f1 and Sox2 binding to ZGA group genes exceeded the average of all groups by a factor of 4 (Fig. 2B). Using the MBT expression gene group independently defined by Aanes *et al.* (23), we confirmed our results obtained for the ZGA group genes (fig. S7). Thus, Pou5f1 and Sox2 preferentially bind to regulatory regions of genes most steeply activated at ZGA.

To address the functional importance of Pou5f1 and SoxB1 for ZGA, we analyzed expression changes in ZGA group genes in response to Pou5f1 and Sox2 overexpression. For identification of directly regulated targets, post-MBT translation was inhibited with cycloheximide (CHX). In *MZspg* mutants, Pou5f1 overexpression strongly activated most ZGA group genes, whereas Sox2 overexpression alone did not (Fig. 2C). However,

in wild-type embryos with endogenous Pou5f1 present, Sox2 overexpression hyperactivated most ZGA group genes (fig. S6). In the other 29 expression groups, no global expression changes were observed upon Pou5f1 overexpression (Fig. 2C). To investigate whether endogenous Pou5f1 is necessary for ZGA, we analyzed expression changes in maternal-only (*Mspg*) and maternal-zygotic (*MZspg*) Pou5f1 mutants. *Mspg* mutants fertilized with wild-type sperm started endogenous Pou5f1 expression at 3 hpf. Most of the ZGA group genes at 4 hpf already had higher expression levels in *Mspg* than in *MZspg* embryos (Fig. 2D). However, *Mspg* embryos showed lower levels of expression of ZGA group genes than wild-type embryos (Fig. 2E), revealing that maternally derived Pou5f1 available pre-MBT contributes to this activation. Similar increases were not observed in the other 29 groups (Fig. 2, D and E, bottom), which demonstrates that Pou5f1 is selectively required pre- and post-MBT for fast activation of most ZGA genes.

To test for overlapping functions of Pou5f1 and SoxB1, we compared phenotypes of SoxB1 quadruple morpholino knockdown (QKD) (13) in wild-type, *MZspg* (Fig. 2, F and G, and fig. S8, A to D), and *Mspg* (fig. S8, E and F, and supplementary text) mutants. Expression onset of the SoxB1 and Pou5f1 combined ZGA group target *klf2a* (table S5) was retarded in *MZspg* SoxB1-QKD relative to *MZspg* or wild-type SoxB1-QKD embryos (Fig. 2, F and G). Thus, SoxB1

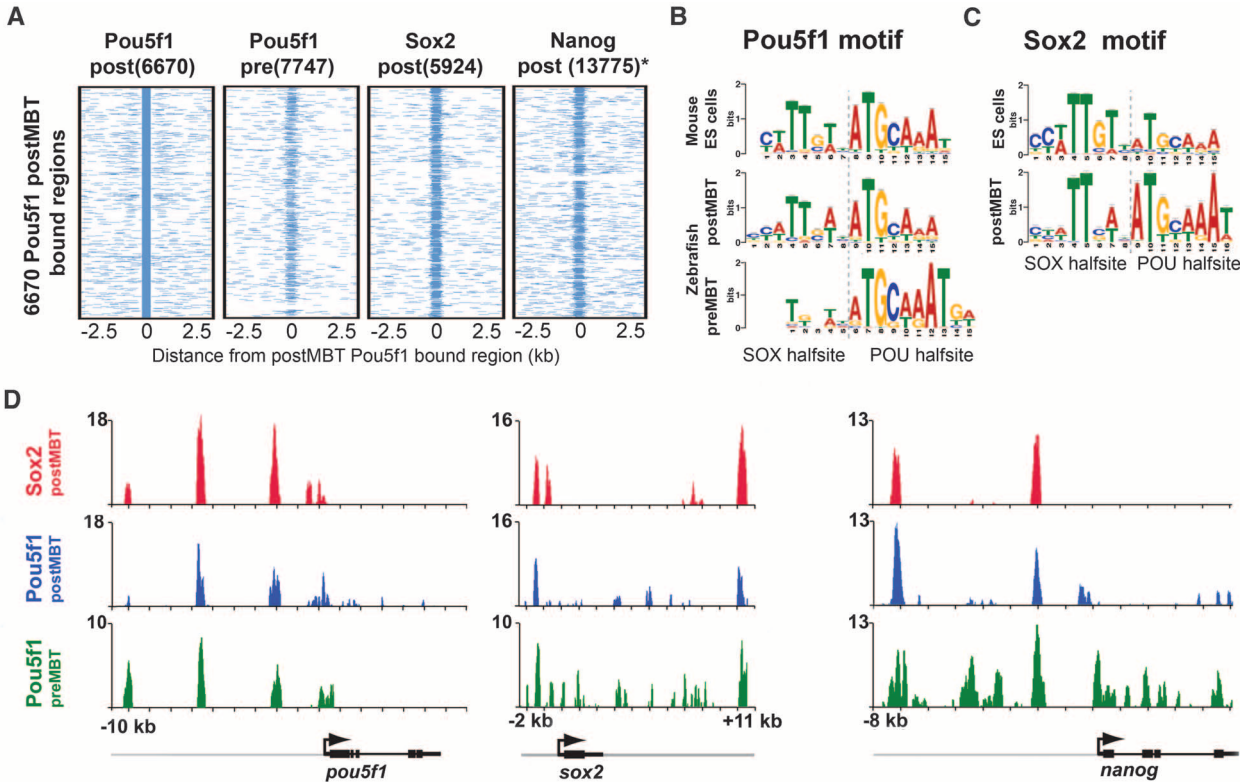


Fig. 1. Characterization of Pou5f1 and Sox2 binding regions and target genes. (A) Region map shows colocalization of Pou5f1 post-MBT ChIP-Seq peaks with pre-MBT Pou5f1, post-MBT Sox2, and post-MBT Nanog peaks; * from (10). Analyzed 5-kb regions are centered on Pou5f1 peaks 320

base pairs long. (B and C) Top-scoring Pou5f1 (B) and Sox2 (C) zebrafish motifs compared with the respective mammalian motifs. (D) Colocalization of Pou5f1 and Sox2 at the regulatory regions of *pou5f1*, *sox2*, and *nanog*; the y axis indicates ChIP-Seq reads per base.

and Pou5f1 both are required for steep up-regulation of *klf2a* expression. However, SoxB1-QKD did not worsen the MZ*spg* or M*spg* embryonic phenotype (Fig. 2G and fig. S8, E and F) at stages preceding gastrulation. These findings indicate that although SoxB1 transcription factors are necessary for fine-tuning expression onset of certain genes, they have no Pou5f1-independent functions essential for embryonic survival during the first several hours after MBT.

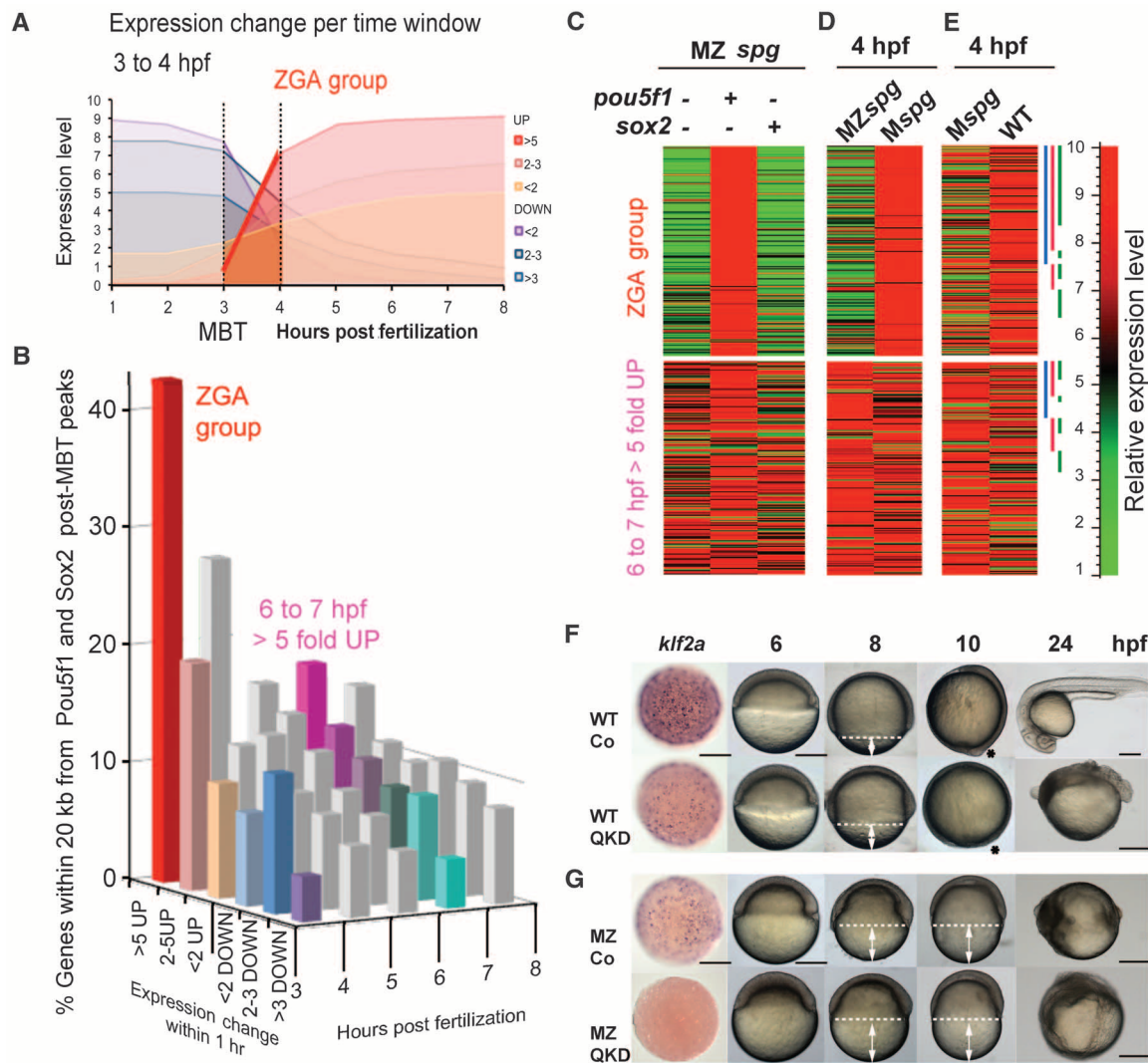
The steep ZGA gene group up-regulation suggests involvement of additional mechanisms preparing for robust activation. Recent studies have revealed several candidate mechanisms, including recruitment of RNA polymerase II (Pol II) and modification of the chromatin landscape at regulatory regions before the actual start of transcription [reviewed in (24)]. To investigate whether these features occur at Pou5f1 and Sox2 binding regions, we compared positions of pre- and

post-MBT Pou5f1 and post-MBT Sox2 binding with pre-MBT, MBT, and post-MBT epigenetic chromatin modification data (25) (Fig. 3A, fig. S9, tables S6 to S8, and supplementary text). Trimethylated histone H3 Lys⁴ (H3K4me3) active chromatin marks and Pol II are enriched at Pou5f1 and Sox2 binding regions before MBT and during later stages (Fig. 3 and fig. S10). Genes premarked with Pol II before MBT at Pou5f1 and Sox2 binding regions (table S9) encode zygotic proteins with key regulatory functions in the early embryo (table S10), including components of major signaling pathways such as *bmp2b*, *dkk1*, *sdf2*, *lefty1*, and *lefty2*. Because Pol II does not have its own DNA-binding specificity, it may be recruited to SOX-POU binding sites by maternal Pou5f1 protein.

High co-occupancy with multiple transcription factors is a characteristic feature of Zelda binding sites in *Drosophila* (6, 8). Therefore, we

investigated whether binding of other transcription factors may also be facilitated at SOX-POU binding regions. We examined co-occupancy of post-MBT Pou5f1 and Sox2 sites with Nanog (10) and three germ layer-specific developmental transcription factors (Mtx2, Smad2, and Ntla) using published zebrafish ChIP-seq or ChIP-on-chip post-MBT data (10, 26, 27). Similar to their orthologs in ES cells, Pou5f1, Sox2, and Nanog tended to colocalize at the same sites (Fig. 1A, fig. S5, C to F, fig. S11, A and B, and supplementary text). Binding regions of Mtx2, Smad2, and Ntla also colocalized with those of Pou5f1 and Sox2 (fig. S11, C to E, and table S1). To analyze the contribution of Pou5f1 and SoxB1 to transcription of Ntla target genes, we combined the published Ntla gene regulation network (26) with Pou5f1 and Sox2 binding and loss-of-function data (13, 21). Pou5f1 or Sox2 bound to promoters or enhancers of most Ntla targets (fig. S11 and

Fig. 2. Pou5f1 activates the earliest zygotic genes. (A) Explanatory chart visualizing the classification of transcripts by expression change per 1-hour time window (the y axis shows arbitrary units). Six exemplified expression profiles are based on level and direction of regulation in the 3- to 4-hpf time window; 162 genes up-regulated by a factor of >5 were defined as ZGA group. (B) Percentage of Pou5f1 and Sox2 targets among the 30 gene groups clustered by expression change per time window. (C to E) Relative expression levels of ZGA group genes (top) and 6- to 7-hpf group up-regulated by a factor of 5 (bottom) in microarray experiments. Each row represents one microarray probe, each column one experimental condition. Genes were sorted according to ChIP-seq Pou5f1 or Sox2 binding, as shown by blue (post-MBT Pou5f1), red (Sox2), and green (pre-MBT Pou5f1) vertical lines. Maximal expression level was set to 10 (color code at right). (C) Pou5f1, but not Sox2,



induces expression of ZGA group genes in MZ*spg*. (D) ZGA genes are up-regulated in M*spg* relative to MZ*spg*. (E) ZGA genes are up-regulated in wild type (WT) relative to M*spg*. (F and G) SoxB1 QKD phenotype (13) in WT (F) and MZ*spg* (G). Scale bars, 0.1 mm. Leftmost column shows whole-

mount in situ hybridization for *klf2a* at 4.7 hpf, animal view. Other columns show live embryos at indicated ages; lateral views, animal pole up. White dotted line, epiboly border; white arrow, free yolk; *, position of tailbud.

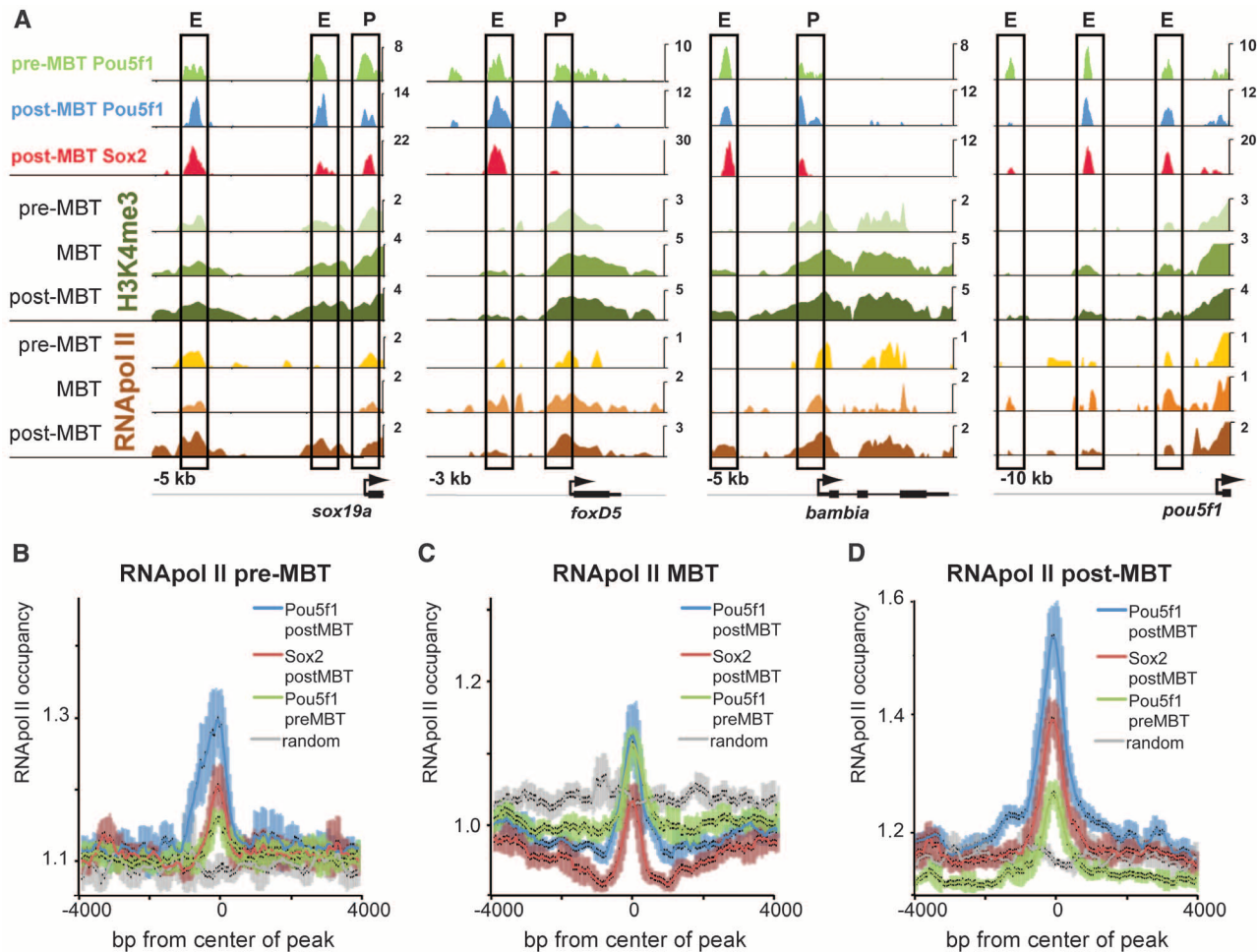


Fig. 3. Pou5f1 and Sox2 binding regions are already enriched in H3K4me3 and Pol II before MBT. (A) Pre- and post-MBT Pou5f1, post-MBT Sox2, Pol II binding, and H3K4me3 modifications at four loci. ChIP-seq data for Pou5f1 and Sox2 are shown in reads per base. For H3K4me3 and Pol II, log₂ Ma2C signal is shown for stages indicated; data from (25). E, enhancer; P, promoter; x axis indicates distance (kb) from TSS. (B to D) Distribution of Pol II across genomic regions centered on the Pou5f1 pre-MBT peaks (green), Pou5f1 post-MBT peaks (blue), Sox2 peaks (red), or background (gray), at indicated stages (analysis in 50-bp steps; shading denotes SD of eight neighboring regions).

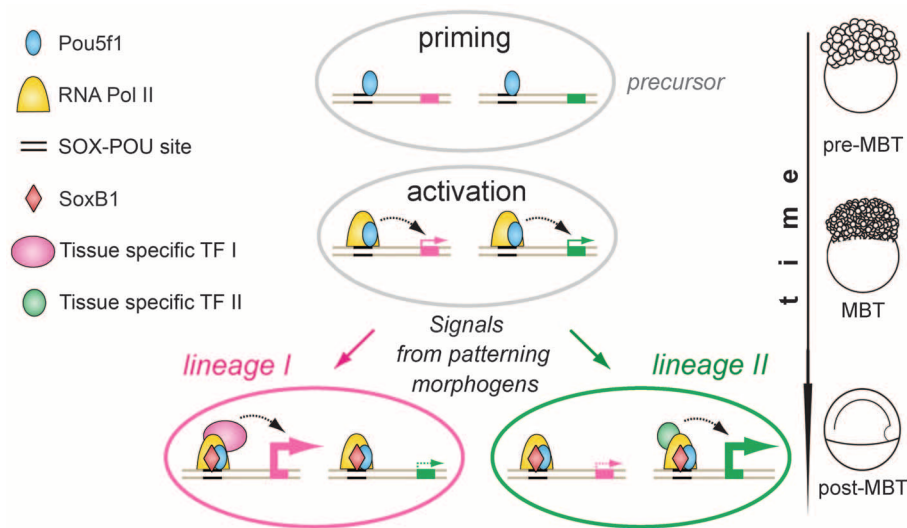


Fig. 4. Priming-activation-timing model of ZGA activators during early development of zebrafish. Before ZGA, Pou5f1 binds to the SOX-POU “priming sites” and attracts Pol II to ensure robust activation at ZGA. After ZGA, SoxB1 proteins co-occupy SOX-POU sites; both factors cooperate with developmental regulators to ensure precise transcriptional timing and proper level of expression.

table S1). The input of Pou5f1 expression was essential for 11 of 28 Ntla target genes in the network; similarly, the input of SoxB1 expression was essential for 6 of the 28 Ntla target genes (fig. S12).

The emerging zebrafish ZGA mechanisms are remarkably similar to those in *Drosophila*. Zelda binds to specific motifs in the enhancers of early-expressed developmental control genes (3–5) to facilitate binding of other transcription factors to these sites by keeping chromatin in the open state (6, 7). Zelda in combination with patterning factors coordinates temporal and spatial expression of early gene batteries (6–8). Homologs of Zelda or transcription factors with similar function have not been reported outside the insect clade. Our results reveal that Pou5f1 functions in a similar fashion as a key ZGA activator involved in priming and activation of the earliest zygotic genes (Fig. 4). At early post-ZGA stages, SoxB1 transcription factors co-occupy SOX-POU binding regions together with Pou5f1 and feed into regulatory cascades involved in regional specification of the embryos,

ensuring that multiple genes have precisely timed transcription starts and sufficient levels of activation (21).

The ancestral roles of the mammalian pluripotency control factors Pou5f1 and Sox2 during the early development of nonmammalian vertebrates have long been a mystery (28). Our results show that the composition of post-MBT Pou5f1 and Sox2 binding sites, co-occupancy with Nanog, their chromatin state, and Pol II binding are similar in zebrafish embryos and mammalian ES cells (fig. S13 and supplementary text). Thus, the ancestral function of the pluripotency factors is zygotic gene activation and developmental timing control in the early vertebrate embryo. In a sense, it is a first major reprogramming event from transcriptionally silent cleavage-stage cells to pluripotent post-MBT blastomeres. In this context, Pou5f1 and Sox2 contribute to all main embryonic regulatory pathways. Considered together with the orthology of target gene sets, the zygotic priming-activation-timing mechanism may have evolved to control the cell pluripotency state in mammalian development.

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Supplementary Materials

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Molecular Basis of Tubulin Transport Within the Cilium by IFT74 and IFT81

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Intraflagellar transport (IFT) of ciliary precursors such as tubulin from the cytoplasm to the ciliary tip is involved in the construction of the cilium, a hairlike organelle found on most eukaryotic cells. However, the molecular mechanisms of IFT are poorly understood. Here, we found that the two core IFT proteins IFT74 and IFT81 form a tubulin-binding module and mapped the interaction to a calponin homology domain of IFT81 and a highly basic domain in IFT74. Knockdown of IFT81 and rescue experiments with point mutants showed that tubulin binding by IFT81 was required for ciliogenesis in human cells.

Cilia are microtubule-based organelles that function in motility, sensory reception, and signaling (1). Ciliary dysfunction results in numerous diseases and disorders commonly known as ciliopathies. Intraflagellar transport (IFT) is involved in cilium formation (2, 3) but also

functions in other cellular processes, such as the recycling of T cell receptors at the immune synapse (4). IFT relies on kinesin-2 and IFT-dynein molecular motors moving along the microtubule-based axoneme of cilia (5–7) and on the IFT complex, which contains at least 20 different protein subunits. Although ~600 proteins are known to reside in the cilium (8), we know very little about how they are recognized as ciliary cargo by the IFT machinery (9–11).

To identify potential cargo-binding sites on the IFT complex, we carried out bioinformatical and biochemical screening and identified conserved domains that were not required for IFT complex formation. We reasoned that such domains could protrude from the IFT particle-core structure and would thus be in a prime position for cargo rec-

ognition. The two IFT core proteins IFT74 and IFT81 were found to possess N-terminal domains (IFT74N and IFT81N) that were not required for IFT complex formation or stability (fig. S1). Whereas IFT81N is highly conserved in sequence and predicted to be a folded domain, IFT74N was likely to be disordered and was highly basic with an isoelectric point (pI) > 12 (fig. S2). To characterize the properties of IFT74N and IFT81N, we purified recombinant *Homo sapiens* (Hs) IFT81N, *Chlamydomonas reinhardtii* (Cr) IFT81N, and a truncated HsIFT74/81 heterodimeric complex (fig. S3) (IFT74N alone degraded rapidly and could not be purified) and determined the crystal structure of CrIFT81N (Fig. 1, A to C; fig. S4, A to D; and table S1). The crystal structure revealed that IFT81N adopts the fold of a calponin homology (CH) domain with unexpected structural similarity to the kinetochore complex component NDC80 with microtubule (MT)-binding properties (12). Given that the cilium consists of a MT-based axoneme, IFT of large quantities of tubulin is required for cilium formation (13). We thus tested the tubulin-binding properties of HsIFT81N using affinity pull-downs (Fig. 1D and fig. S4E) and microscale thermophoresis (MST) with unpolymerized bovine α -tubulin (Fig. 1, E and F). HsIFT81N bound tubulin with a dissociation constant (K_d) of 16 μ M via a highly conserved, positively charged surface patch, which was enhanced 18-fold by IFT74N (Fig. 1G and fig. S3). Because this result was unexpected, we also carried out MT sedimentation assays and electron microscopy (EM) to visualize IFT81 or IFT74/81 bound to MT (fig. S5). The IFT74/81 complex,

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but not IFT81N, at low μM concentration cosedimented with MT during ultracentrifugation (fig. S5, D and E) and decorated MT (fig. S5F). Thus, the tubulin-binding module is formed by the IFT74/81 complex rather than by IFT81N alone.

To dissect the binding mode in the IFT74/81: $\alpha\beta$ -tubulin complex, samples were prepared from MT and unpolymerized $\alpha\beta$ -tubulin lacking the highly acidic C-terminal tails, often referred to as E-hooks (12) (fig. S5A). $\alpha\beta$ -tubulin lacking E-hooks had similar affinity for IFT81N as intact tubulin (fig. S5, B and C), which suggested that IFT81N recognizes the globular domain of $\alpha\beta$ -tubulin with no substantial interaction with the E-hooks. IFT74/81 displayed robust MT binding in sedimentation assays, which was, however, reduced to background levels in the absence of the β -tubulin E-hook (fig. S5E). Thus, IFT81N appears to bind the globular domain of tubulin to provide specificity, and IFT74N recognizes the β -tubulin tail to increase affinity (Fig. 1H).

To examine the role of tubulin binding by IFT74/81 in a cellular system, we transiently expressed Flag-HsIFT81 or Flag-HsIFT81 ΔN in human RPE-1 cells and induced formation of primary cilia either by treatment with 0.5 μM cytochalasin D (Fig. 2) (14) or serum starvation (fig. S6, A and D). In these experiments, centrioles were visualized by staining for CAP350 and cilia by staining for the small guanosine triphosphatase Arl13b

or acetylated tubulin. We detected both Flag-HsIFT81 and Flag-HsIFT81 ΔN at the tip of the primary cilium and, to a minor extent, also along the axoneme (fig. S6A), suggesting that IFT81N is not required for the transport of IFT81 within the primary cilium. Remarkably, however, the expression of Flag-HsIFT81 ΔN had a strong negative impact on the extent of ciliogenesis (fig. S6, B to D), suggesting that excess IFT81 ΔN caused a dominant-negative effect, presumably through formation of IFT complexes unable to bind tubulin.

To further investigate the function of the tubulin-binding domain of IFT81 in ciliogenesis, we carried out small interfering RNA (siRNA)-rescue experiments. siRNA-mediated depletion of IFT81 (fig. S6E) strongly reduced the percentage of ciliated cells (Fig. 2), which could be rescued by coexpression of an siRNA-resistant full-length IFT81, as expected. In contrast, none of the IFT81 mutants deficient in tubulin binding in vitro (HsIFT81mut¹ and HsIFT81mut²) compensated fully for the depletion of endogenous IFT81. Whereas expression of the deletion mutant (HsIFT81 ΔN) or the mutant with reduced tubulin-binding ability (HsIFT81mut¹) resulted in partial rescue, expression of HsIFT81mut², in which the entire tubulin-binding patch was mutated, completely failed to rescue the siRNA-mediated knockdown of IFT81 (Fig. 2B). Thus, the entire negative effect on cilium formation by IFT81 depletion was reca-

pitulated with a specific tubulin-binding-deficient mutant.

The fact that IFT81 ΔN formed stable IFT core complexes (fig. S1) suggested that the ciliogenesis phenotype was because of reduced tubulin binding and not a general failure of IFT. To rule out whether mutation of the IFT81N CH domain resulted in general IFT deficiency, we turned to the unicellular protozoan parasite *Trypanosoma brucei*, where IFT has been well studied (15), and tested the effect of IFT81N CH-domain disruption on IFT. Yellow fluorescent protein (YFP)-tagged but otherwise normal IFT81 or mutant IFT81 (IFT81_{I46D,L47D}, Dm) where the CH domain was unfolded (fig. S7A) were expressed at wild-type levels. One of the two *IFT81* alleles was replaced with either YFP::IFT81 or YFP::IFT81Dm, leaving one WT *IFT81* allele unaltered (fig. S7B). Both the localization and the IFT speed of IFT81 and IFT81Dm were similar to that observed for other IFT proteins as judged by live-cell imaging and kymographic analysis (Fig. 3 and movies S1 and 2) (15). Thus, IFT81N is not required for IFT complex assembly or normal IFT in vivo, which corroborates that the ciliogenesis phenotype observed upon IFT81N CH-domain mutation (Fig. 2) was indeed because of tubulin-binding deficiency.

Axonemal precursors such as tubulin are added to the tip of the cilium in a length-dependent man-

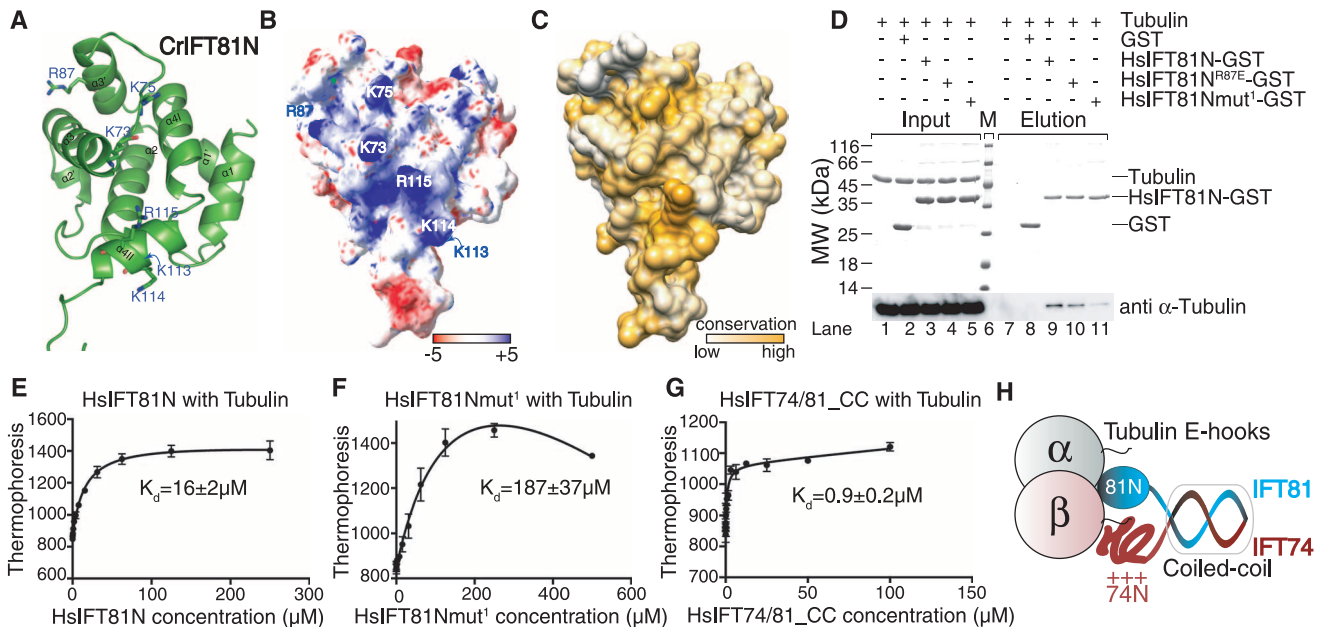


Fig. 1. IFT81 and IFT74 form a tubulin-binding module. (A) Cartoon representation of the crystal structure of CrIFT81N domain, with conserved lysines and arginines implicated in tubulin binding shown as sticks. (B) Electrostatic surface potential of IFT81N displaying the positively charged patch with the residues labeled according to the HsIFT81 sequence. (C) Surface conservation of IFT81N demonstrates that the basic patch is well conserved among different species (also see fig. S2). (D) Tubulin binding evaluated by glutathione (GST) affinity pull-down of bovine $\alpha\beta$ -tubulin using glutathione S-transferase (GST)-HsIFT81N. Whereas tubulin does not bind the GST alone and is not pulled down by GST alone, a substantial portion is pulled down by GST-HsIFT81N, demonstrating binding. Whereas the single-point mutation R87E does not

strongly impair binding, the K73K75/EE double mutant (mut¹) results in reduced amounts of pulled-down tubulin, indicating reduced binding. (E) Quantification of tubulin binding to untagged HsIFT81N by microscale thermophoresis reveals a K_d of 16 μM . (F) The HsIFT81N mut¹ has drastically reduced binding with a K_d of 187 μM , showing that the basic patch is required for tubulin binding. (G) Microscale thermophoresis titration of tubulin with truncated HsIFT74/81 complex reveals a K_d of 0.9 μM . The curves in (E), (F), and (G) are calculated for three independent experiments, and the error bars represent the mean $\pm$ SD. (H) The experiments shown in (D) to (G), along with the data in fig. S5, suggest a model in which IFT81N recognizes the globular domain of tubulin, providing specificity, and IFT74N binds the acidic tail of β -tubulin, providing increased affinity.

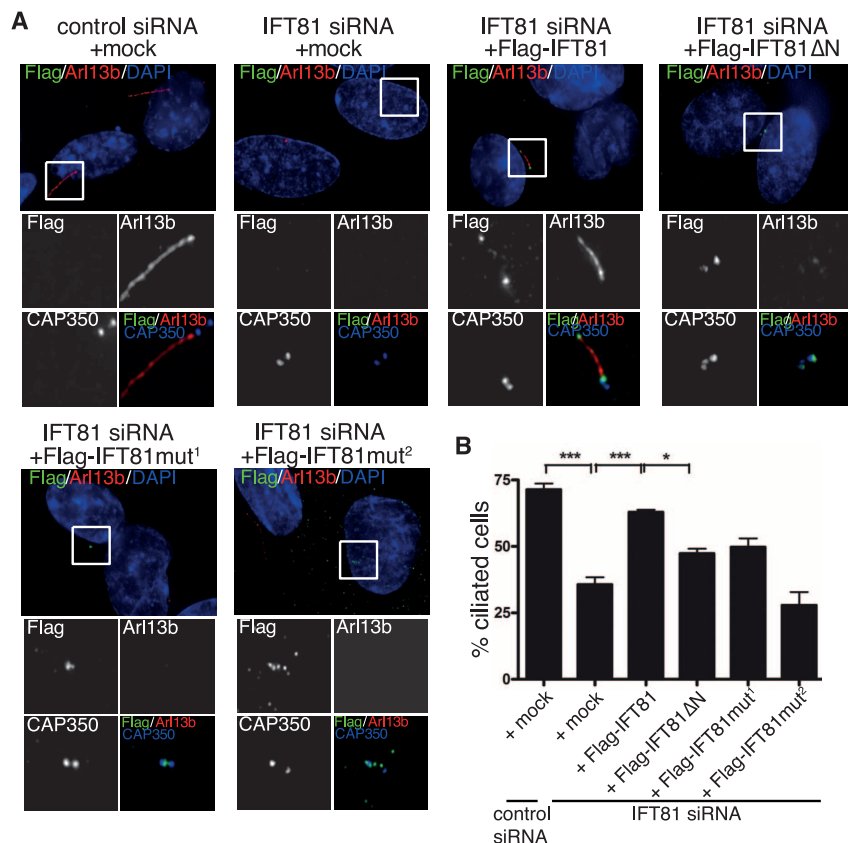


Fig. 2. Tubulin binding by IFT81 is required for ciliogenesis in human cells. (A) Transient expression of Flag-IFT81, but not the tubulin-binding-deficient IFT81 mutants (in green), rescues the ciliogenesis defect after IFT81 siRNA knockdown. Primary cilia formation was induced by 0.5 μ M cytochalasin D and detected by antibody to Arl13b (in red). CAP350 (in blue, inset images only) was used to visualize centrosomes. Mut¹ and Mut² are K73K75/EE and K73K75K113K114R115/EEEE tubulin-binding mutants, respectively. Scale bar, 5 μ m. (B) Quantification of the rescue experiment shown in (A). $n = 3$ independent experiments; statistical analyses by one-way analysis of variance.

ner (16, 17). The removal of tubulin from the axonemal tip, on the other hand, appears to be constant, with no dependence on cilium length (17, 18). These observations inspired the balance-point model, in which the length of a mature cilium is the result of equal delivery and removal rates for axonemal precursors (17, 19). Furthermore, the concentration of tubulin in the cytoplasm influences ciliogenesis and cilium length in mammalian cells (20). Based on the measured affinity between IFT74/81 and tubulin ($K_d = 0.9 \mu$ M) (Fig. 1G), we calculated the fraction of IFT complexes bound to $\alpha\beta$ -tubulin as a function of tubulin concentration (Fig. 4A). Because the cellular tubulin concentration is estimated to be in the low μ M range (21) and tubulin expression is induced at the onset of ciliogenesis (22), the IFT74/81:tubulin affinity is optimal for regulating cilium length via tubulin transport (Fig. 4B). The prediction is thus that most IFT complexes are loaded with tubulin during early stages of ciliogenesis, whereas lower occupancies are found during steady-state cilium length (Fig. 4), which agrees well with previously obtained data demonstrating that tubulin transport in full-length cilia yields only faint traces on kymographs, likely due to low tubulin occupancy on IFT complexes (13). During cilium growth, both anterograde IFT complex concentration and tubulin binding are negatively correlated with cilium length, resulting in a decreasing assembly rate as the cilium approaches steady-state length.

Here, we have shown that the two core IFT proteins IFT74 and IFT81 form a tubulin-binding module required for ciliogenesis, which suggests a role of IFT74/81 in the transport of tubulin within cilia. The fact that the high-affinity binding of tubulin occurs only for the IFT74/81 complex and

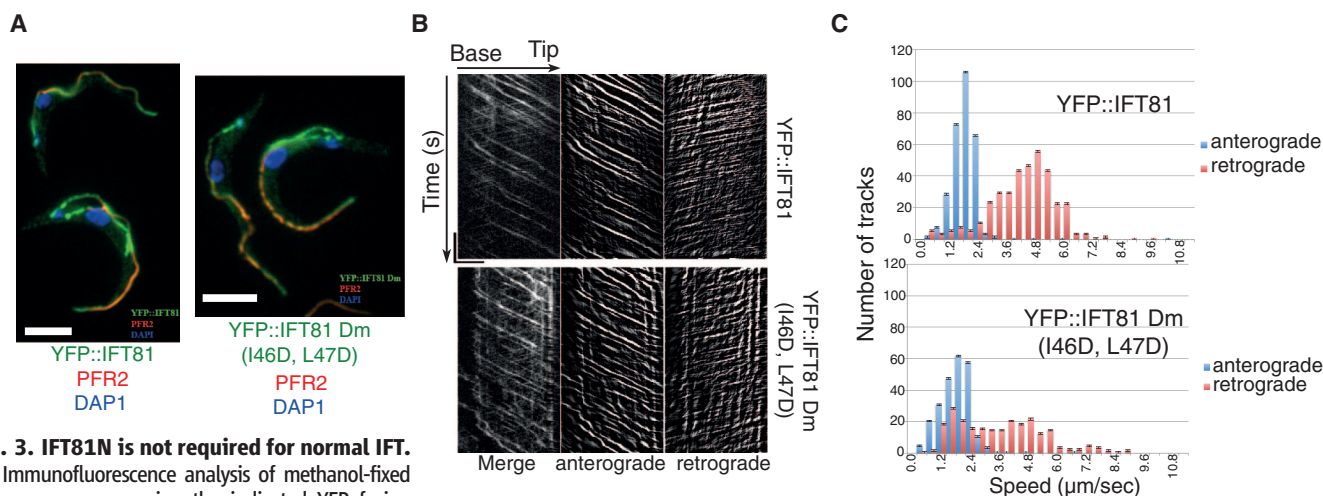


Fig. 3. IFT81N is not required for normal IFT. (A) Immunofluorescence analysis of methanol-fixed trypanosomes expressing the indicated YFP fusion proteins from the endogenous locus stained with an antibody to green fluorescent protein (GFP) (green) and with the antibody to PFR2 L8C4 to visualize the flagellum (red). The left panel corresponds to a control strain expressing YFP::IFT81 and the right panel to the mutant YFP::IFT81Dm, where the IFT81N CH domain is unfolded. Scale bar, 5 μ m. (B) Kymograph generation and separation of anterograde and retrograde tracks. Kymographs were extracted from videos of cells expressing YFP::IFT81 (movie S1) or YFP::IFT81Dm (movie S2). Panels show the complete kymograph, anterograde events, and retrograde events (from left to right). The x axis corresponds to the length of the flagellum (horizontal scale bar,

5 μ m) and the y axis to the elapsed time (vertical scale bar, 5 s). (C) Quantitation of the kymograph analysis shown in (B). Anterograde (blue) and retrograde velocity (red) distribution of IFT particles are calculated from cells expressing YFP::IFT81 and YFP::IFT81Dm. The kymographic analysis reveals robust anterograde trafficking with a speed of $1.75 \pm 0.55 \mu$ m/s for YFP::IFT81 ($n = 294$ tracks from 15 cells) and $1.68 \pm 0.72 \mu$ m s⁻¹ for YFP::IFT81Dm ($n = 244$ tracks from 15 cells). These values are in line with those reported for anterograde movement of GFP-IFT52 (15). Curiously, retrograde transport was slowed down in the case of YFP::IFT81Dm, where a second population of relatively slow trains was detected.

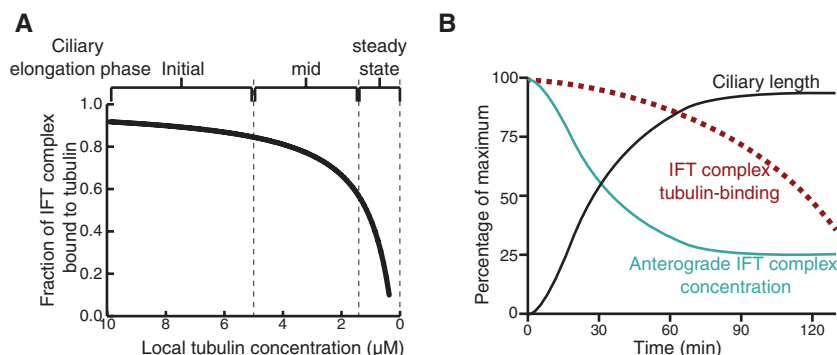


Fig. 4. Model for tubulin transport and ciliary length control. (A) Fraction of IFT complex bound to tubulin at varying tubulin concentrations is plotted using the equation $O_{IFT} = [Tub]/(K_d + [Tub])$. O_{IFT} is the fraction of IFT bound to tubulin, K_d is the binding constant that is experimentally determined in this study as $0.9 \mu M$, and $[Tub]$ is the local concentration of free tubulin at the base of the cilium. (B) From the point of initiation of flagellar regeneration, the relationship between the ciliary length, the concentration of anterograde IFT particles, and O_{IFT} is plotted.

not for IFT81 alone could help ensure that tubulin cargo only binds in the context of properly assembled IFT complexes. Because tubulin constitutes the backbone of all cilia, it makes sense that the tubulin has a dedicated cargo-binding site on the IFT core complex (23). We hypothesize that, although abundant ciliary cargo proteins such as tubulin may undergo IFT via dedicated transport modules, less abundant ciliary proteins are likely to compete for generic cargo-binding sites on the IFT complex.

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Supplementary Materials

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Crystal Structure of MraY, an Essential Membrane Enzyme for Bacterial Cell Wall Synthesis

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MraY (phospho-MurNAc-pentapeptide translocase) is an integral membrane enzyme that catalyzes an essential step of bacterial cell wall biosynthesis: the transfer of the peptidoglycan precursor phospho-MurNAc-pentapeptide to the lipid carrier undecaprenyl phosphate. MraY has long been considered a promising target for the development of antibiotics, but the lack of a structure has hindered mechanistic understanding of this critical enzyme and the enzyme superfamily in general. The superfamily includes enzymes involved in bacterial lipopolysaccharide/teichoic acid formation and eukaryotic N-linked glycosylation, modifications that are central in many biological processes. We present the crystal structure of MraY from *Aquifex aeolicus* (MraY_{AA}) at 3.3 Å resolution, which allows us to visualize the overall architecture, locate Mg²⁺ within the active site, and provide a structural basis of catalysis for this class of enzyme.

Bacteria maintain their cell shapes at different osmotic pressures by using the mesh-like layers of the cell wall to surround and

stabilize the membrane. The cell wall of both Gram-negative and Gram-positive bacteria is composed of peptidoglycan, a cross-linked polymer

of carbohydrates and amino acids. Because biosynthesis of peptidoglycan is a critical process for bacteria, it has been a major target for antibiotics (1, 2). Peptidoglycan biosynthesis involves three main stages. First, the peptidoglycan precursor UDP-N-acetylmuramoyl (MurNAc)-pentapeptide (L-Ala-γ-D-Glu-diaminopimelic acid/L-Lys-D-Ala-D-Ala) is synthesized in the cytosol. Second, this hydrophilic precursor is attached to a carrier lipid, and the lipid-linked precursor is flipped across the membrane to the periplasm. Third, peptidoglycan precursors are polymerized to form the cell wall.

MraY, or phospho-MurNAc-pentapeptide translocase, is an integral membrane protein responsible for the second stage of peptidoglycan

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biosynthesis. *MraY* catalyzes the transfer of phospho-MurNAc-pentapeptide from UDP-MurNAc-pentapeptide to the lipid carrier undecaprenyl phosphate (C_{55} -P), yielding undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide, also known as Lipid I (Fig. 1A). This *MraY*-mediated membrane step is Mg^{2+} -dependent and essential for bacterial viability (3, 4). Pharmacologically, *MraY* has long been known as a promising target for the development of new antibiotics because it is the target of five different classes of natural product inhibitors with antibacterial activity as well as bacteriolytic protein E from bacteriophage phiX174 (1, 5–11).

MraY belongs to a subfamily of the polyprenylphosphate *N*-acetyl hexosamine 1-phosphate trans-

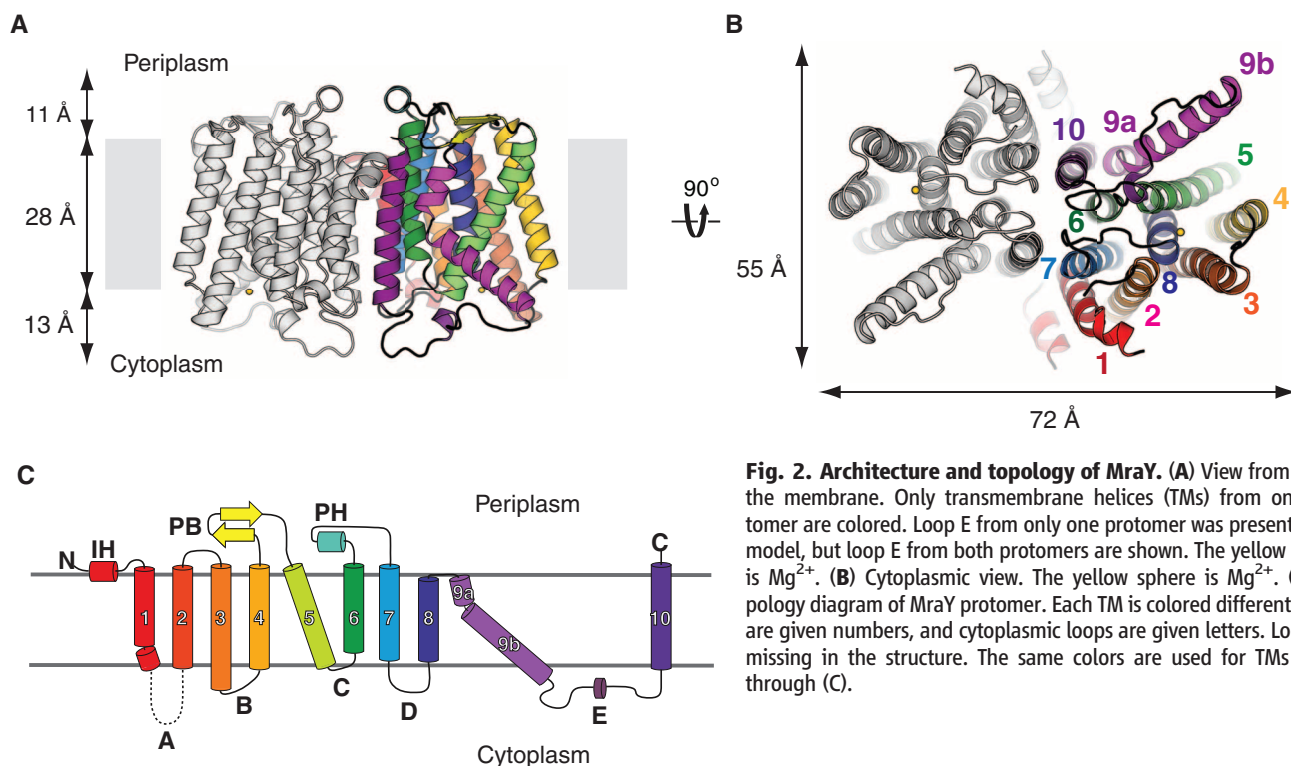
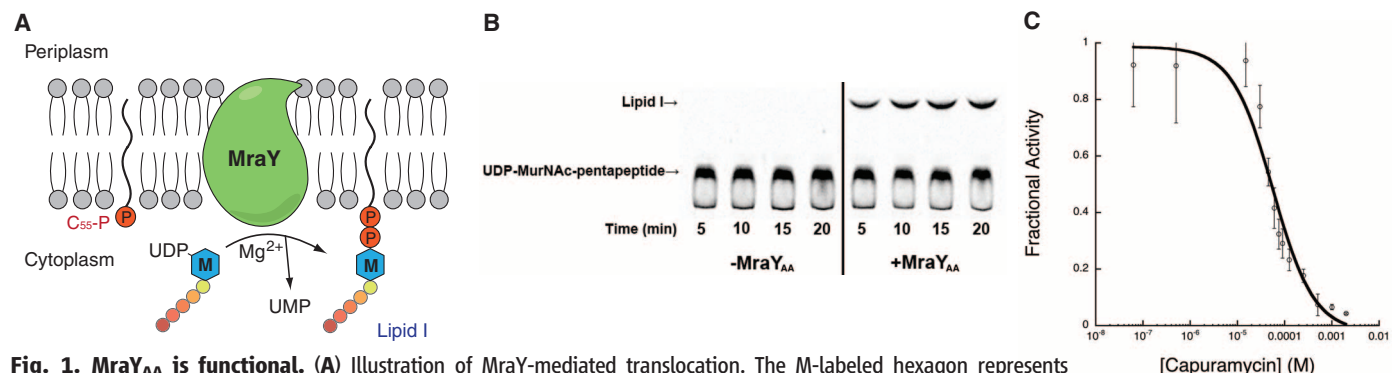
ferase (PNPT) superfamily. The PNPT superfamily includes the *MraY*, *WecA*, *TagO*, *WbcO*, *WbpL*, and *RgpG* enzyme families, which are responsible for the synthesis of cell envelope polymers such as the O-antigen and teichoic acid in bacteria, and the GPT (UDP-GlcNAc:dolichol-P GlcNAc-1-P transferase) enzyme family, which is responsible for the committed step of N-linked glycosylation in eukaryotes (12). The structural basis of enzyme function has been elusive because no structure from this enzyme superfamily is available.

For structural and functional studies, recombinant *MraY* from *Aquifex aeolicus* (*MraY*_{AA}) was expressed, purified, and crystallized (supplementary materials, materials and methods). Single anomalous dispersion phasing was performed

by using a selenomethionine-substituted *MraY*_{AA} crystal (fig. S1). The final model was refined with good geometry (table S1 and S2).

A thin-layer chromatography (TLC)-based translocase assay (13) shows that recombinant *MraY*_{AA} can catalyze the transfer of phospho-MurNAc-pentapeptide to the carrier lipid C_{55} -P (Fig. 1B). Endogenous *Escherichia coli* *MraY* does not contribute appreciably to the observed activity (fig. S2). We also used the translocase assay to show that *MraY*_{AA} could be inhibited by the *MraY*-specific natural product inhibitor capuramycin with a median inhibitory concentration (IC_{50}) value of about 56 μ M (Fig. 1C) (7, 9).

*MraY*_{AA} crystallizes as a dimer in the asymmetric unit. The twofold axis of the dimer is



perpendicular to the putative membrane plane and parallel to one of the crystallographic two-fold axes (Fig. 2A). At the center of the dimer interface is an oval-shaped tunnel. The tunnel is surrounded mostly by hydrophobic amino acids

and is large enough to accommodate lipids (Fig. 2B). An unbiased electron density map shows that two elongated electron density peaks, too long to be detergent molecules, are intertwined within the dimer interface (fig. S3). The overall dimen-

sions of the dimer are about 72 Å across the long axis and 55 Å across the short axis when viewed from the cytoplasmic side (Fig. 2B). Each protomer contains 10 transmembrane helices (TM1 to TM10), an interfacial helix (IH), a periplasmic β

Fig. 3. The active site of *MraY_{AA}*.

(A) Conservation mapping on the *MraY_{AA}* structure. Sequence conservation is colored on one protomer with a gradient from magenta (absolutely conserved) to cyan (least conserved) based on 28 *MraY* sequences (fig. S5). The structure is rotated ~45° toward the reader relative to Fig. 2A. The arrow indicates the active site cleft.

(B) Mutation mapping on the *MraY_{AA}* structure based on the studies of Al-Dabbagh *et al.* (16). Mutations leading to pronounced functional effects are shown in stick representation. Among these mutations, amino acid residues that appear to be important for catalysis and active site structural maintenance are colored orange and cyan, respectively.

(C) Mutational studies of putative active-site residues of *MraY_{AA}* using the same translocase assay as Fig. 1B. The specific activities of mutants were normalized to that of wild type (Data are shown as means ± SD, *n* = 3 experiments).

(D) Active site Mn^{2+} (Mg^{2+})-binding site. Anomalous difference Fourier density for Mn^{2+} , shown in green mesh contoured at 5.2σ, is calculated from a data set collected from a crystal grown in the presence of Mn^{2+} without Ni^{2+} by using phases derived from the model without the metals. Another anomalous difference Fourier density peak for Mn^{2+} , shown in red mesh

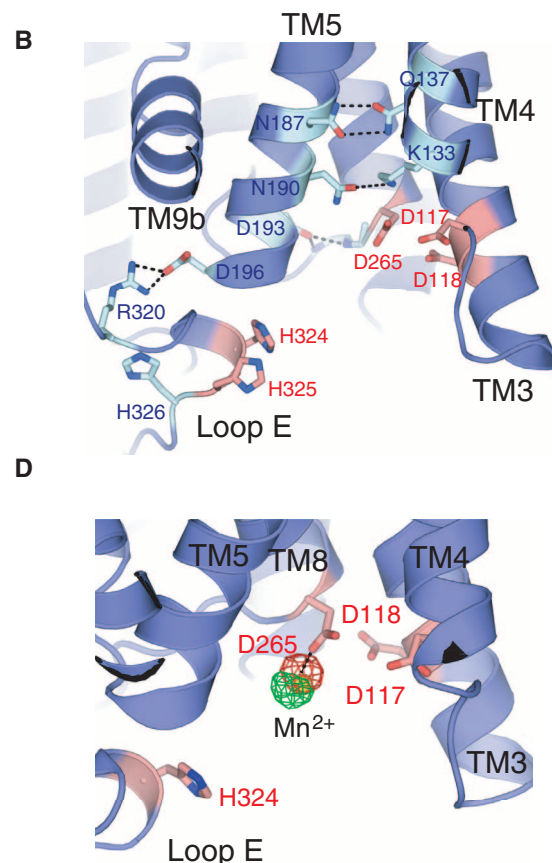
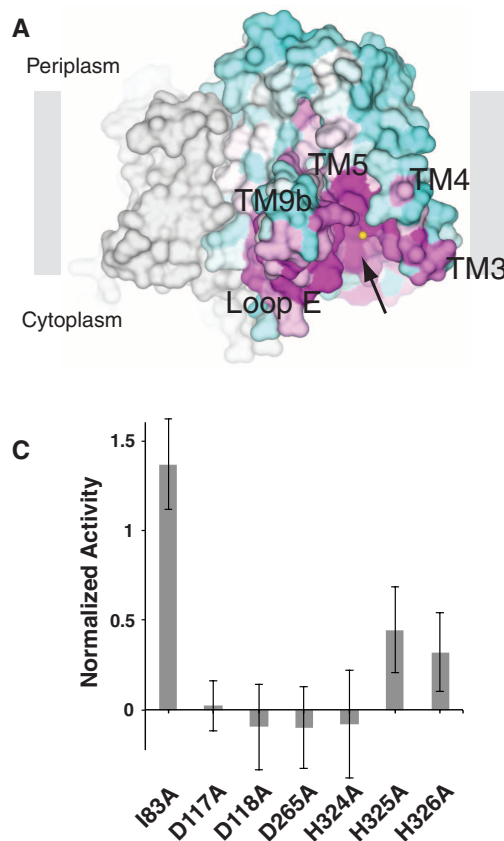


Fig. 4. Putative substrate-binding sites. **(A)** Zoomed-in view of TM9b, loop E (the HHH motif). The structure is rotated 90° toward the reader relative to Fig. 3A. TM9b/loop E is shown in sausage representation, with the thicker region more conserved (magenta) and the thinner region less conserved (cyan). Side chain of highly conserved amino acids on TM9b and loop E as well as the

contoured at 3.4σ, was calculated from a data set collected from a crystal soaked with Mn^{2+} in the presence of Ni^{2+} by using phases derived from the model without the metals.

catalytic residue D265 are shown in stick representation. Mg^{2+} and Ni^{2+} are shown as yellow and green spheres, respectively. **(B)** A hydrophobic groove connected to the active site is delineated with dashed lines on the *MraY_{AA}* surface illustration. Mg^{2+} is shown as a yellow sphere, and D117 is colored red. Protomers are colored blue and gray.

hairpin (PB), a periplasmic helix (PH), and five cytoplasmic loops (loop A to loop E) (Fig. 2C). Both the N- and C-termini of the structure are located on the periplasmic side, which is consistent with previous topological studies (14). TM9 breaks into two helical fragments (TM9a and TM9b), with TM9b displaying a substantial bend (~50° relative to the membrane normal) in the middle of the membrane. This bending causes TM9b to protrude ~20 Å into the lipid membrane away from the rest of structure (Fig. 2, A and B). This protrusion is buttressed by interactions with TM5. TM9b—together with TM3, TM4, and TM8—surrounds TM5 and thus generates a cleft around the inner leaflet membrane region of TM8 (Fig. 2B).

To test the oligomeric status of *MraY_{AA}*, we performed cross-linking studies in both detergent micelles and lipid membranes. For detergent-solubilized *MraY_{AA}*, we performed the experiment using the nonspecific cross-linker disuccinimidyl suberate (DSS), and for membrane-embedded *MraY_{AA}*, we performed structure-guided disulfide cross-bridge experiments (fig. S4). The results show that *MraY_{AA}* forms a dimer both in detergent micelles and in the membrane. Consistent with this observation, a recent bacterial two-hybrid study indicated that *MraY* enzymes in *Caulobacter crescentus* interact with each other (15).

To gain functional insight, we mapped sequence conservation onto the *MraY_{AA}* structure (Fig. 3A and fig. S5). The highest conservation is localized around the cleft formed by the cytoplasmic and inner-leaflet membrane regions of TM3, TM4, TM8, and TM9b (Fig. 3A). Recent mutational studies of *Bacillus subtilis* *MraY* showed that 14 invariant polar/charged amino acid residues are essential as evidenced by both *in vivo* functional complementation assay and *in vitro* enzymatic assay (16). Most of these residues are localized in the cleft, suggesting that this region serves as the active site (Fig. 3B). On the basis of our mutational mapping, we predicted that three invariant aspartate residues (Asp¹¹⁷, Asp¹¹⁸, and Asp²⁶⁵) conserved throughout the entire PNPT family and two invariant histidine residues (His³²⁴ and His³²⁵) in the *MraY* family are catalytically important because they do not appear to be involved in maintaining the putative active site structure (Fig. 3B). We individually mutated each of these three invariant aspartate as well as three histidine residues (His³²⁴, His³²⁵, and His³²⁶) and found that mutation of any of the three aspartate residues and one histidine residue (His³²⁴) resulted in nearly complete loss of activity of *MraY_{AA}*, whereas mutating a nonconserved residue (Ile⁸³) away from the active site (on TM2) did not reduce catalytic activity (Fig. 3C). We observed that there is no appreciable difference in stability upon mutation. Thus, at least three acidic residues and one histidine residue (Asp¹¹⁷, Asp¹¹⁸, Asp²⁶⁵, and His³²⁴) in the active site are important for catalysis.

It had been assumed that the residues corresponding to Asp¹¹⁷ and Asp¹¹⁸ in *MraY_{AA}* are involved in Mg²⁺ coordination based on sequence

similarity to the Mg²⁺-binding motif (DDXXD/N) of prenyl transferases (17), but this assumption was challenged recently (16). To identify Mg²⁺ within the active site of the structure, we performed anomalous scattering studies using Mn²⁺-substituted *MraY_{AA}* crystals (experimental details are available in the supplementary materials, materials and methods). Anomalous difference Fourier density maps calculated from both Mn²⁺-soaked and Mn²⁺-co-crystallized crystals displayed peaks at the active site near Asp²⁶⁵ (Fig. 3D). The Mn²⁺ directly interacts only with Asp²⁶⁵ but not with Asp¹¹⁷ or Asp¹¹⁸. An F_O-F_C OMIT electron density map from the native data at 3.3 Å shows that there are unassigned density peaks within interacting distance of the Mg²⁺, which are probably involved in Mg²⁺ coordination (fig. S6).

From sequence conservation and topological studies, it was predicted that *MraY* contains five conserved cytoplasmic loops (fig. S5) (3). Substantial portions of the loops correspond to the inner-leaflet membrane region of TM helices in the structure. The fifth predicted loop is composed of TM9b and the loop connecting TM9b to TM10 (loop E) (Fig. 4A and fig. S5). This region shows subfamily-specific sequence conservation within the PNPT superfamily (18, 19), and it was predicted that it has a role in the specific recognition of sugars. This is consistent with a study of *WecA*, which showed that this region may interact with sugar nucleotides (18).

Within the two protomers of the asymmetric unit, only one (chain A) shows well-resolved electron density for loop E (fig. S7). Loop E is ~23 amino acids long and is composed of several basic residues followed by a stretch of sequence (PXHHHXXG) that is highly conserved within the *MraY* family (hereafter termed “HHH motif”). The HHH motif includes a short stretch of helical segment followed by a loop (Fig. 4A). Two Ni²⁺ ions, verified through anomalous scattering studies, are coordinated by the short helical segment, one by the first two histidines (His³²⁴ and His³²⁵) and the second by the third histidine (His³²⁶) and Glu³²⁸ (fig. S8). The translocase assay with the concentrations of Mg²⁺ (100 mM) and Ni²⁺ (10 mM) used for crystallization resulted in full activity, suggesting that the structure of Ni²⁺-bound loop E reflects a physiologically relevant conformation (fig. S9).

TM9b is an amphipathic helix substantially tilted and protruding toward the lipid membrane and, together with loop E (HHH motif), abuts the active site, thus making the active site cleft deeper (Figs. 3 and 4A). Highly conserved polar and charged amino acids on TM9b and loop E (the HHH motif) are localized and pointed toward a region near the active site Mg²⁺, which is consistent with the previous notion that this region is the binding site for the hydrophilic substrate UDP-MurNAc-pentapeptide. An F_O-F_C OMIT map shows a τ-shaped density peak threaded and trapped in the gap between TM9b and loop E (fig. S10).

Bouhss and colleagues proposed that Asp¹¹⁷ (Asp⁹⁸ in *B. subtilis* *MraY*) interacts with and

deprotonates the phosphate moiety of C₅₅-P. Asp¹¹⁷ is one of the three catalytically important acidic residues in the active site (16). The structure of *MraY_{AA}* is in line with the prediction that Asp¹¹⁷ is the binding site for the phosphate group of C₅₅-P because it is surrounded by the conserved residues Lys¹²¹, Lys¹³³, and Asp²⁶⁵ as well as the Mg²⁺ ion (fig. S11). The surface representation of *MraY_{AA}* reveals that there is an inverted-U-shaped groove surrounding TM9b that extends into the active site where Asp¹¹⁷ is located (Fig. 4B). Because C₅₅-P is longer than the depth of the membrane, and is elastic owing to the polyisoprenyl group, the inverted-U-shaped groove within the membrane region of the enzyme could perhaps serve as the binding site for the hydrophobic substrate C₅₅-P (Fig. 4B).

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the model. B.C.C. performed the cross-linking studies and prepared all the protein for functional assays. J. Z. and R.A.G. performed the translocase assays (J.Z. performed all the mutational studies, and R.A.G. performed capuramycin and divalent metal experiments) under the guidance of P.Z. D.-Y.K. synthesized capuramycin under the guidance of

J.H. Z.G. performed mass spectrometry characterization. B.C.C. and S.-Y.L. wrote the paper.

Supplementary Materials

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Materials and Methods

Figs. S1 to S11
Tables S1 and S2
References (20–27)

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β CaMKII in Lateral Habenula Mediates Core Symptoms of Depression

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The lateral habenula (LHb) has recently emerged as a key brain region in the pathophysiology of depression. However, the molecular mechanism by which LHb becomes hyperactive in depression remains unknown. Through a quantitative proteomic screen, we found that expression of the β form of calcium/calmodulin-dependent protein kinase type II (β CaMKII) was significantly up-regulated in the LHb of animal models of depression and down-regulated by antidepressants. Increasing β -, but not α -, CaMKII in the LHb strongly enhanced the synaptic efficacy and spike output of LHb neurons and was sufficient to produce profound depressive symptoms, including anhedonia and behavioral despair. Down-regulation of β CaMKII levels, blocking its activity or its target molecule the glutamate receptor GluR1 reversed the depressive symptoms. These results identify β CaMKII as a powerful regulator of LHb neuron function and a key molecular determinant of depression.

Major depressive disorder (MDD), one of the most prevalent and disabling mental disorders, is characterized by low mood, loss of motivation, feelings of despair, and an inability to feel pleasure, also known as anhedonia (1). Modern views on the cause of MDD suggest that the neural activities of specific brain circuits are altered in response to external stimuli, such as stress, as a result of maladaptive molecular and cellular changes (2, 3). Recently, the lateral habenula (LHb), a nucleus that relays information from the limbic forebrain to multiple monoamine centers, has emerged as a key brain region in aversive behaviors and the pathophysiology of depression (4–10). LHb neurons are activated by aversive emotional cues, including stress, disappointment, fear, or anticipation of a negative reward (4–6). Consistently, neuroimaging studies have identified heightened habenula activity in the depressed state (11–13). Furthermore, synaptic activity and spike output of LHb neurons were enhanced in animal models of depression (14). However, what molecular mechanisms

underlie these aberrant cellular processes in LHb and how depression-inducing stimuli lead to these changes are yet to be determined.

We conducted an unbiased, mass spectrometry-based, quantitative proteomic screening to compare habenular protein expression of wild-type controls with that of congenitally learned helpless (cLH) rats, a well-accepted model of depression (15). cLH rats were selectively bred for the phenotype of learned helplessness (16). They displayed significantly reduced lever pressing to escape foot shocks, which was reversed by chronic antidepressant treatment (imipramine, injected intraperitoneally 10 mg per kg of body weight, for 14 days) (Fig. 1A). cLH rats also showed increased (longer) immobility in the forced swim test (Fig. 1A), another animal model of depression that reflects behavioral despair (17), although basic motor and cognitive functions are normal (15).

We microdissected the habenula of cLH and wild-type control rats and extracted protein for quantitative proteomic analysis based on ¹⁵N stable isotope labeling (Fig. 1B) (18). To reduce sample complexity, we extracted the membrane fraction and analyzed three independent sets of samples (figs. S1 to S3 and table S1). The β form of calcium/calmodulin-dependent protein kinase type II (β CaMKII) was significantly up-regulated in the habenula of cLH rats (1.9 times the β CaMKII of wild-type control, $P = 0.01$) (Fig. 1C). Other CaMKII family isoforms were also examined: α CaMKII levels varied widely across samples, although an increasing trend was observed; δ CaMKII remained unchanged; and γ CaMKII showed a 1.3-fold increase ($P = 0.0013$) (fig. S4). As β CaMKII is more enriched in the brain than γ CaMKII (19, 20), we focused on this CaMKII isoform. Secondary

validation by Western blot analysis confirmed that β CaMKII in the membrane fraction of cLH habenular protein samples increased to 1.86 times those of the controls ($P = 0.03$) (Fig. 1D). In contrast, the β CaMKII protein level in cLH hippocampal samples decreased (63% of control, $P = 0.048$) (Fig. 1E), probably because of neural atrophy and spine loss in the hippocampus associated with depression (21, 22). β CaMKII mRNA in cLH habenula increased to 1.37 times the amount in controls ($P = 0.04$), as measured by quantitative real-time PCR (QPCR) (Fig. 1F), which suggested that transcriptional regulation contributed to at least part of the change in the amount of protein. Immunohistochemical staining of habenular brain slices revealed that the CaMKII protein increased in the lateral part of the habenula (LHb) (fig. S5).

We further examined β CaMKII in two additional depression models, acute learned helplessness, induced by repeated inescapable and uncontrollable foot shocks (16), and chronic mild stress, induced by prolonged exposure to unpredictable mild stressors (23). The amounts of β CaMKII were also significantly increased in these two stress paradigms (Fig. 1G). Furthermore, chronic antidepressant treatment with imipramine, which reversed the depressive phenotypes of cLH rats (Fig. 1A), caused significant down-regulation of β CaMKII protein in the habenula of cLH rats (Fig. 1H).

We next investigated to what extent the change in β CaMKII in LHb is necessary or sufficient to cause depressive behaviors or whether it is merely a biological marker of the depressive state. We first constructed viral vectors [adeno-associated virus 2 (AAV2)] to overexpress β - or α CaMKII in the LHb of wild-type rats and mice and tested the effects on various depression models (Fig. 2, A to C). We used the ubiquitin promoter to drive ubiquitous, high-level gene expression, as LHb neurons are almost uniformly glutamatergic (24). To estimate the level of overexpression, we injected CaMKII-expressing viruses into one side of the habenula. Fourteen days after injection, CaMKII levels in the injected side were 4.4 (± 0.6)-fold for β CaMKII and 4.6 (± 0.9)-fold for α CaMKII of the noninjected side, respectively (fig. S6). We then injected virus into both sides of LHb and allowed expression for 10 days before behavioral testing of depression (Fig. 2, B and C, and fig. S7).

Overexpression of β CaMKII in the LHb of unstressed mice produced significantly increased immobility time and decreased latency to immobility onset in the forced swim test, compared with the injected control (Fig. 2D). Locomotor activities of these mice were not significantly different (fig. S8), which indicated that the immobility

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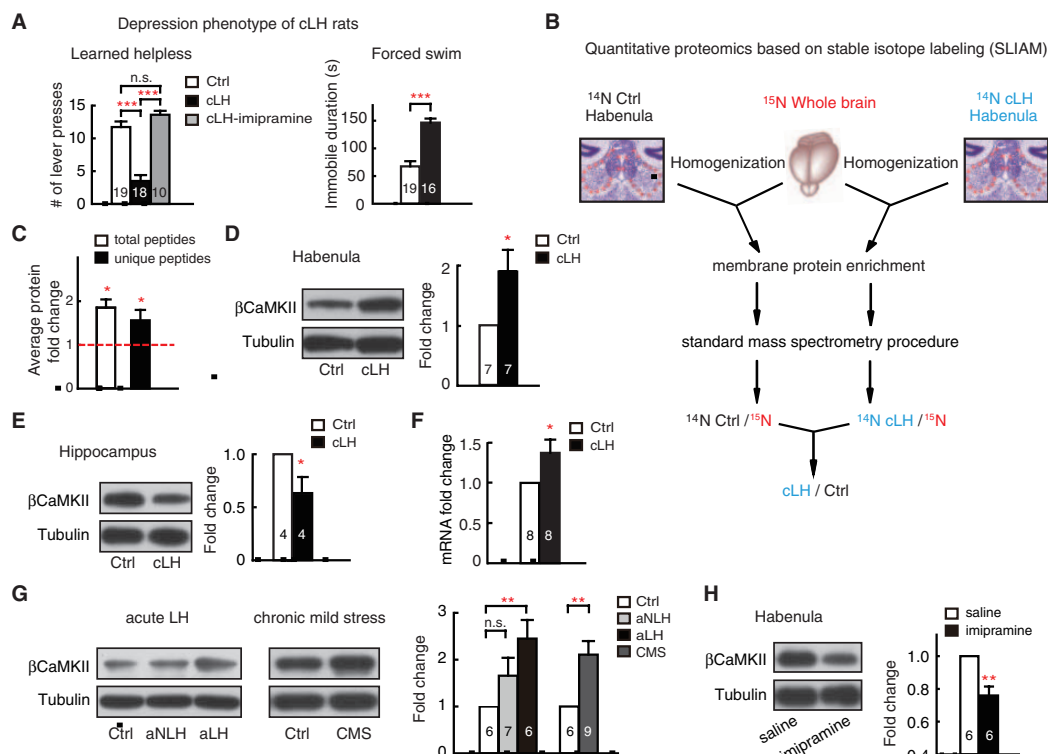
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Fig. 1. β CaMKII is up-regulated in the LHb of animal models of depression.

(A) Depression phenotypes of cLH rats. Numbers in the bars indicate the number of animals used. Note that, in the LH test, the maximal number of bar presses is 15. (B) Experimental outline of the high-throughput quantitative proteomics based on stable isotope labeling. Briefly, habenula of (red dotted area) unlabeled (^{14}N) wild-type control (WT) or cLH rats were dissected, homogenized, and mixed in a 1:1 ratio with total brain homogenate from a ^{15}N -labeled rat. Membrane fraction was enriched, and a 100- μg protein sample was used for standard mass spectra analysis. We calculated the $^{14}\text{N}/^{15}\text{N}$ ratio for each identified peptide. Peptide ratios for each protein were then compared between cLH and control sample. For details, see methods. (C) Proteomic analysis of β CaMKII, based either on total peptides or unique peptides (peptides not shared by other CaMKII family members) identified in three independent proteomic runs. (D and E) Western blot analysis showing change of β CaMKII in membrane fraction of habenula (D) or hippocampus (E) of cLH rats. Tissue amounts of tubulin were used as loading control. Protein expression was normalized by control amount. (F) QPCR analysis of β CaMKII mRNA in habenula. (G) β CaMKII increase in acute learned helpless and chronic mild stress (CMS) depression models. aLH and aNLH were rat groups subjected to LH stress that did (aLH), or did not (aNLH), display LH symptom. (H) Western



blot analysis showing amount of β CaMKII in the membrane fraction of habenula of cLH rats treated with saline or antidepressant imipramine. Data are means $\pm$ SEM. We used two-tailed Student's t tests for two-group comparison, one-way analysis of variance (ANOVA) with Bonferroni post hoc analysis for multiple-group comparison. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control group; n.s., not significant.

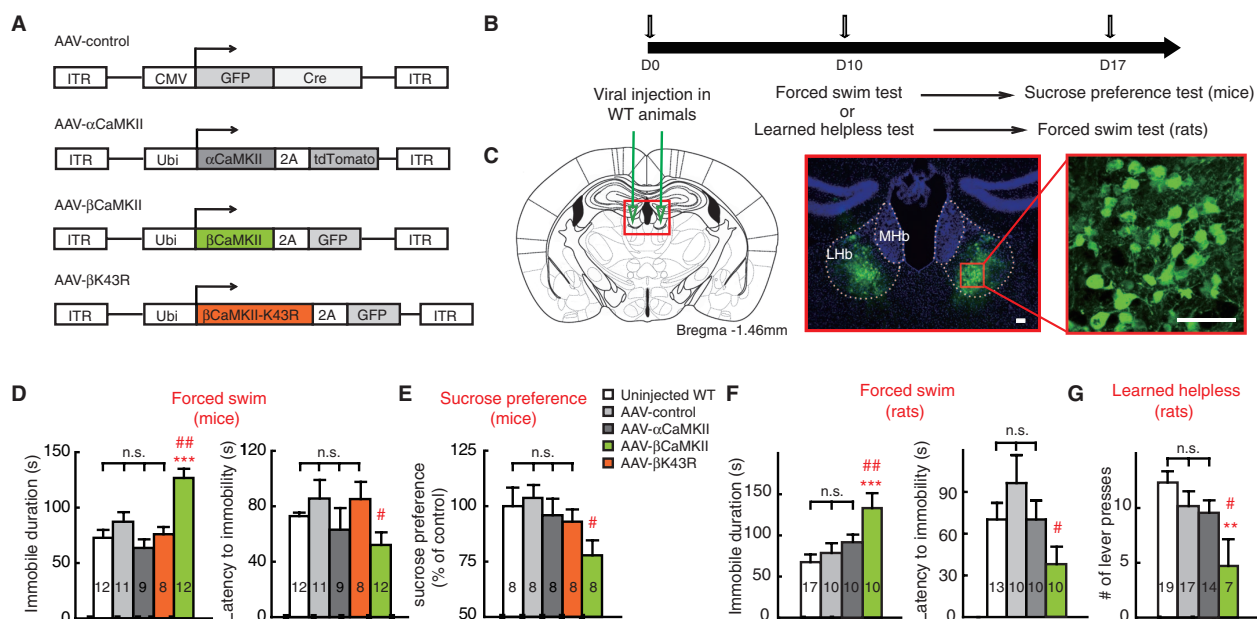


Fig. 2. Overexpression of β CaMKII but not α CaMKII in LHb caused depressive-like behaviors in both mice and rats. (A) Schematics of AAV vectors engineered to overexpress a control construct, β CaMKII, α CaMKII, or a kinase-dead mutant of β CaMKII. ITR, inverted terminal repeats; CMV, cytomegalovirus promoter; Ubi: ubiquitin promoter; 2A: viral 2A linker peptide allowing translation of multiple unfused proteins. (B) Experimental paradigm for behavioral testing of WT mice or rats. (C) Illustration of

bilateral viral injection of AAV- β CaMKII in mouse LHb (counterstained with antibody against GFP and nuclear marker Hoechst). Scale bars, 50 μm . (D to G) Behavioral effects of expressing various viral constructs in LHb in animal models of depression in mice (D to E) or rats (F and G). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with noninjected WT, # $P < 0.05$, ## $P < 0.01$ compared with AAV-control, one-way ANOVA with Bonferroni post hoc analysis.

was not likely due to motor defects. In addition, β CaMKII overexpression also caused anhedonia, evident from a significant reduction in the preference for the sucrose solution (Fig. 2E). To estimate the minimal infection rate required to produce the depression phenotype, we bilaterally injected an additional group of mice with 1:10 diluted AAV- β CaMKII virus. Unlike the normal injection group (having an infection rate of $38 \pm 3\%$), this sparse injection group (having an infection rate of $5 \pm 0.6\%$) did not exhibit depressive-like phenotypes (fig. S9). Overexpression of α CaMKII, or a control green fluorescent protein (GFP)-Cre construct, at a similar infection rate ($36 \pm 2.5\%$ for α CaMKII, $36 \pm 3\%$ for GFP-Cre) did not cause similar depressive-like effects (Fig. 2, D and E). β CaMKII can act both as a kinase and a structural scaffolding protein at the synapses (25). A kinase-dead version of β CaMKII [in which lysine 43 is replaced by arginine (β K43R)] (26), even when overexpressed similarly to wild-

type β CaMKII (4.6 ± 0.5 -fold, infection rate of $40 \pm 3\%$) (fig. S6), did not cause depressive-like phenotypes (Fig. 2, D and E), which suggested that the kinase function of β CaMKII was required to produce depression. Furthermore, in rats, overexpression of β CaMKII in the LHb significantly increased immobility in the forced swim test and reduced the escape behavior, as indicated by the number of times the bar was pressed to terminate foot shocks in the learned helplessness test (Fig. 2, F and G).

To investigate the cellular mechanism by which β CaMKII overexpression alters LHb neuron activity and function, we performed paired whole-cell patch-clamp recordings on viral-infected and neighboring uninfected LHb neurons in acute brain slices of wild-type rats (Fig. 3A). First, to examine the synaptic property of LHb neurons, we measured the miniature excitatory postsynaptic currents (mEPSCs), which are mediated by the AMPA subtype of glutamate receptors and reflect

individual synaptic responses onto recorded neurons. In noninfected brain slices, neighboring LHb neuron pairs showed highly similar mEPSC frequency, despite heterogeneity across different subregions of the LHb (fig. S10). In neurons infected by AAV- β CaMKII, mEPSC frequency was greatly increased ($351 \pm 51\%$ of neighboring controls, $P = 0.0001$), as was mEPSC amplitude ($130 \pm 10\%$ of controls, $P = 0.007$) (Fig. 3, B and C). In contrast, AAV- α CaMKII infection caused a slight decrease in mEPSC frequency ($81 \pm 9\%$ of controls, $P = 0.08$) and no change in mEPSC amplitude ($87 \pm 5\%$ of controls, $P = 0.16$) (Fig. 3, B and D). The effects on mEPSC frequency largely resembled what has been shown in hippocampal neurons for these two CaMKII isoforms (27). Further, to examine the output of these LHb neurons, we measured the spontaneous spiking rate in a cell-attached configuration. The spiking rate of AAV- β CaMKII-infected neurons increased 3.0-fold compared with neighboring uninfected neurons ($P = 0.0004$)

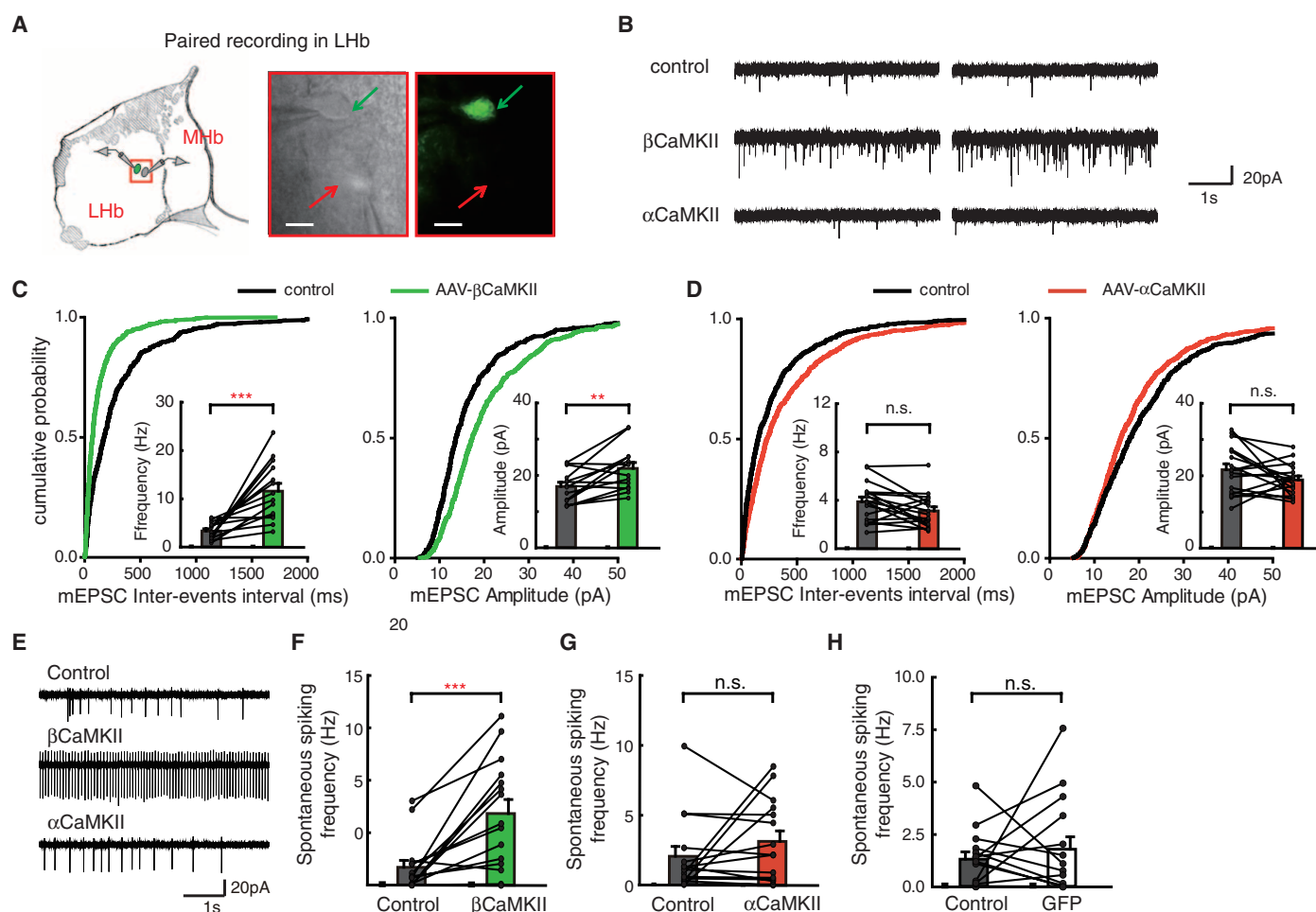


Fig. 3. Overexpression of β CaMKII increased synaptic activity and spike output of LHb neurons. (A) Schematics of paired recording configuration in LHb (left). (Right) Paired patching of a β CaMKII-infected LHb neuron (green arrow) and a neighboring uninfected neuron (red arrow) under transmitted and fluorescent light microscopy. Scale bars, 10 μ m. (B) Example mEPSC traces, measured in a whole-cell configuration, from LHb neurons of control neurons or neurons infected by AAV- β CaMKII or AAV- α CaMKII. (C and D) Cumulative distribution of mEPSC interevents interval and aver-

age frequency (left), or mEPSC amplitude (right) of neurons infected by AAV- β CaMKII (C) or AAV- α CaMKII (D). Each line represents values from a pair of control and neighboring viral-infected neurons. (E) Example traces of spontaneous spiking, measured in a cell-attached configuration, from LHb neurons of control neurons, or neurons infected by AAV- β CaMKII or AAV- α CaMKII. (F to H) Average spontaneous spiking frequency of neurons infected by AAV- β CaMKII (F), AAV- α CaMKII (G), or AAV-GFP (H). ** $P < 0.01$, *** $P < 0.001$, Wilcoxon signed-rank test.

(Fig. 3, E and F), whereas no such change was detected for AAV- α CaMKII-infected ($P = 0.7$) (Fig. 3G), or AAV-GFP-infected neurons ($P = 0.95$) (Fig. 3H).

To determine whether down-regulation of β CaMKII or blockade of β CaMKII functions in LHB reversed depressive phenotypes, we first used RNA interference (RNAi) to reduce (knock down) the amount of β CaMKII protein (Fig. 4A). A previously reported short hairpin RNA specifically targeting the β CaMKII transcript can effectively reduce β CaMKII protein without affecting α CaMKII (28) (Fig. 4B). We then expressed this RNAi form of β CaMKII in AAV virus (AAV- β RNAi) and targeted its expression to the LHB of cLH rats by bilateral stereotactic injection (Fig. 4C). In cLH rats infected by AAV- β RNAi, the immobility time in the forced swim test was markedly reduced ($P < 0.001$), and the escape behavior in the learned help-

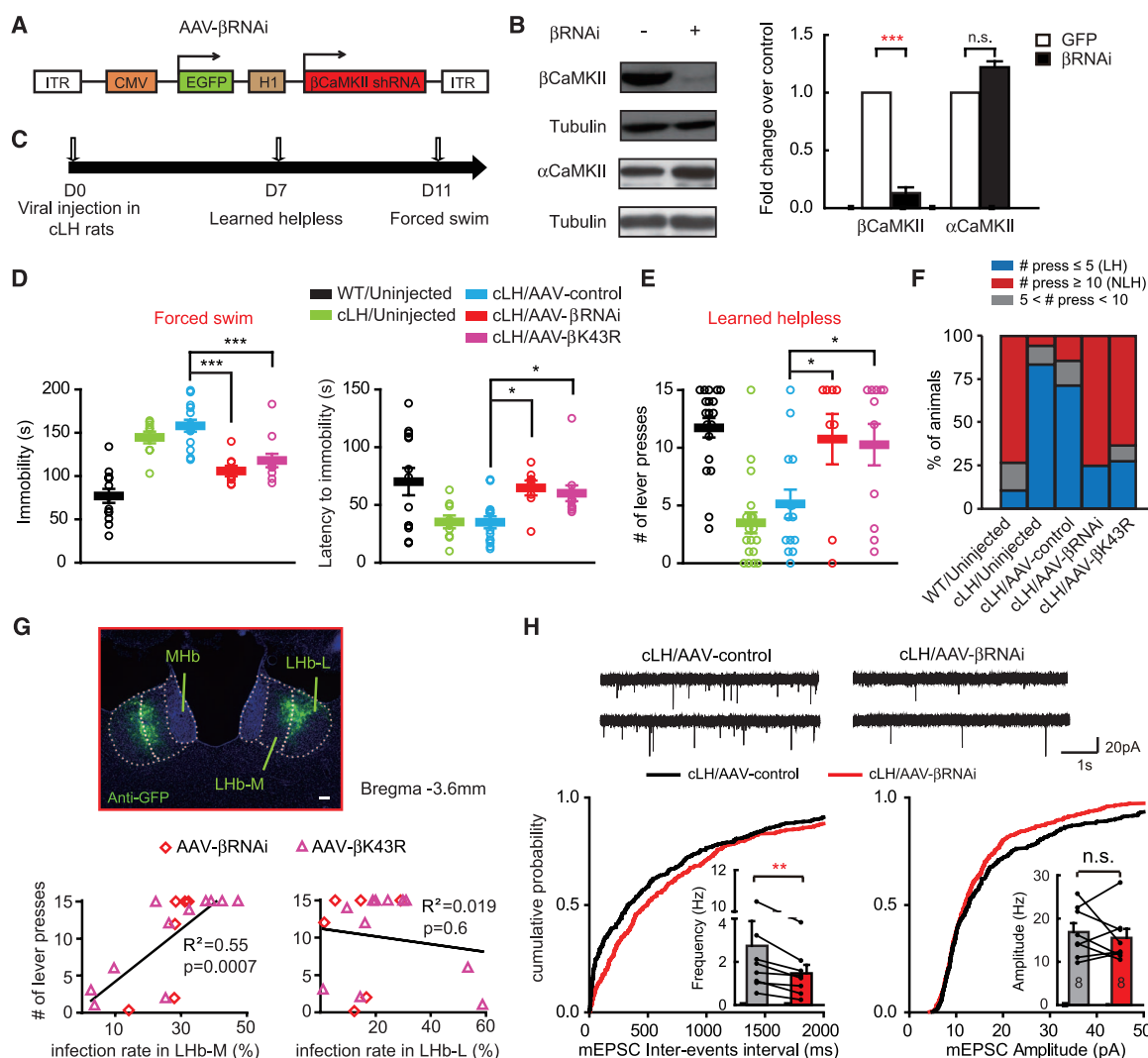
less test significantly increased ($P < 0.05$) (Fig. 4, D and E). The percentage of learned helpless animals (defined as those that pressed the bar less than five times) dropped from 83.3 to 25% (Fig. 4F).

To rule out potential off-target effects by RNAi, we tested a kinase-dead version of β CaMKII, β K43R, which acts as a dominant-negative protein to block endogenous β CaMKII function when overexpressed (26) (Fig. 2A). When we injected AAV- β K43R virus into the LHB of cLH rats, we observed antidepressive effects similar to those seen with AAV- β RNAi in both the forced swim and learned helplessness tests (Fig. 4, D and E). It was noteworthy that an infection rate as low as $17 \pm 3\%$ for AAV- β RNAi or $24 \pm 3\%$ for AAV- β K43R was sufficient to achieve these strong antidepressive effects, which suggested that the LHB neural network has little redundancy and that altering the activity of a relatively small percentage

of LHB neurons is sufficient to ameliorate depression. Thus, we further analyzed infection within subregions of LHB, and found that infection rates in the medial, but not lateral, part of LHB were strongly correlated with the rescuing effects on learned helplessness (Fig. 4G). In the LHB brain slices of cLH rats, neurons infected by the AAV- β RNAi showed significantly reduced frequency of mEPSCs ($53.5 \pm 15.3\%$ of neighboring uninfected controls, $P = 0.008$) (Fig. 4H).

What could be the downstream molecular targets of β CaMKII in mediating the LHB hyperactivity in depression? Up-regulation of β CaMKII increases the synaptic expression and delivery of glutamate receptor GluR1-type AMPAR in cultured hippocampal neurons (29). Thus, we examined the amount of GluR1 in the LHB of cLH rats and found that the membrane fraction of GluR1 was up-regulated ($222 \pm 41.7\%$ of controls,

Fig. 4. Knocking-down of β CaMKII in LHB rescued depression-like phenotypes of cLH rats and reduced synaptic activity of LHB neurons. (A) Schematics of the AAV vector engineered to overexpress an RNAi form of β CaMKII. H1, human H1 promoter. (B) Specific knocking down of β CaMKII but not α CaMKII by the β CaMKII RNAi construct. pSuper- β CaMKII-RNAi construct was cotransfected with AAV- β CaMKII or AAV- α CaMKII plasmid in 293TN cells and expressed for 48 hours before Western analysis. (Left) Representative Western blot. (Right) quantification of knock-down efficiency. (C) Experimental paradigm for behavioral testing of cLH rats infected by virus. (D to F) Behavioral effects of expressing AAV- β RNAi and AAV- β K43R in the LHB of cLH rats in forced swim (D) or learned helplessness test (E and F). * $P < 0.05$, *** $P < 0.001$, one-way ANOVA with Bonferroni post hoc test. (F) Percentage of animals in each category. LH, learned helpless rats with ≤ 5 lever presses; NLH, non-learned helpless rats with ≥ 10 lever presses. (G) Subregional characterization of viral infection. (Top) The division of lateral LHB (LHB-L) and medial LHB (LHB-M) in an AAV- β RNAi virus-infected cLH rat brain slice. Scale bar, 100 μ m. (Bottom) Behavioral response in the learned helplessness test plotted against infection rates in the LHB-L and LHB-M of cLH rats. (H) mEPSCs of AAV- β RNAi-infected LHB neurons



from cLH rats were altered. (Top) Examples of mEPSC traces. Cumulative distribution of mEPSC interevents interval and average frequency (bottom left) or mEPSC amplitude (bottom right) of cLH LHB neurons infected by AAV- β RNAi. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with cLH/AAV-control, Wilcoxon signed-rank test.

$P < 0.01$) (fig. S11A). Conversely, in cLH rats treated with the antidepressant imipramine, the membrane GluR1 level was decreased ($72 \pm 8\%$ of controls, $P = 0.003$) (fig. S11B). To test the role of GluR1 in β CaMKII-mediated depression, we coexpressed β CaMKII with a dominant-negative form of GluR1, GluR1Ct, which blocks the synaptic trafficking of GluR1 (30, 31), using an AAV- β CaMKII-2A-GluR1Ct viral construct. Tests after unilateral injection revealed that β CaMKII exhibited a 3.8-fold and GluR1Ct a 3.7-fold overexpression of endogenous levels (fig. S11C). When this virus was bilaterally injected into the LHb of wild-type mice (infection rate of $37 \pm 2\%$), mice performed normally in both the forced swim and sucrose preference tests (fig. S11, D and E). Therefore, coexpression of GluR1Ct prevented the depressive effects of β CaMKII overexpression.

Here, we identified β CaMKII as a key molecular determinant of habenular hyperactivity and behavioral depression, using a combination of molecular, behavioral, and electrophysiological approaches. Our results point to a model in which stress-induced up-regulation of β CaMKII in the LHb causes more GluR1 insertion into synapses, resulting in increased synaptic efficacy. In addition, β CaMKII may regulate other channels and membrane properties of LHb neurons to enhance spike output, all of which work in concert to cause LHb hyperactivity and lead to enhanced suppression of downstream monoamine centers (fig. S12A). Previous studies have implicated changes in CaMKIIs related to stress and antidepressant response (32–35). However, it was unclear whether these changes are necessary or sufficient for depression etiology, and which brain region or isoform is crucial (36). β CaMKII is about eight times as sensitive to calcium/calmodulin as α CaMKII (37) and has an actin-binding motif that is absent in α CaMKII (38). It will be interesting to pinpoint in the future which feature of β CaMKII renders this isoform-specific function in depression.

An important future question is how depressive stimuli and antidepressants lead to bidirectional changes in β CaMKII levels in the LHb. Aversive emotional stimuli activate LHb neurons (4–6), whereas serotonin boosted by antidepressants suppresses excitatory inputs onto the LHb (7). Thus, it is tempting to speculate that β CaMKII levels are regulated by LHb neural activity. During the onset of depression, prolonged activation of LHb neurons may cause the up-regulation of β CaMKII to reach a threshold level, which can lead to further LHb neural activation. Conversely, antidepressant-caused sup-

pression of the LHb may down-regulate β CaMKII, which can further lower LHb activity. These self-reinforcing feedback processes may eventually drive long-term adaptive changes in emotional states. Whether and how neuronal activity regulates β CaMKII expression awaits further investigation.

In the context of our finding that the medial part of the LHb (LHb-M) is critical for the rescue of learned helplessness behavior (Fig. 4G), it is relevant to note that stress-induced c-Fos activation is relatively confined to the LHb-M (4) and that there were more neurons with higher mEPSC frequency in LHb-M than those in LHb-L (fig. S10A). Further, LHb-M and LHb-L have different circuit wiring, which could contribute to their differential involvement in aversive behaviors and depressive symptoms (fig. S12B). Molecular manipulations in the nucleus accumbens (NAc) specifically mediate anhedonia but not behavioral despair (39). We found manipulation of β CaMKII levels in the LHb affected both of these symptoms, which suggests that LHb may function upstream of NAc in the depression-related circuitry to control multiple aspects of depressive symptoms. Hence, the molecular targets identified in the LHb in this study should provide new insights for therapies that treat both of these core symptoms of depression.

References and Notes

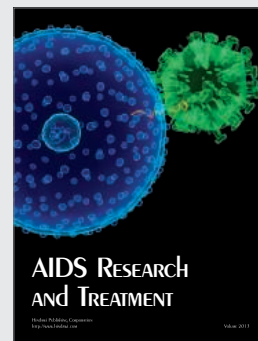
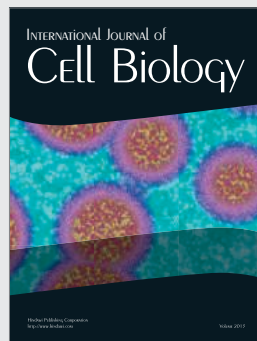
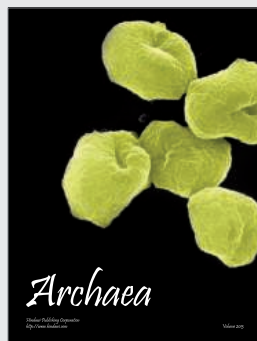
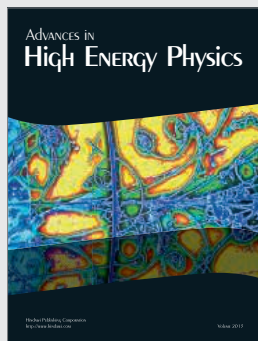
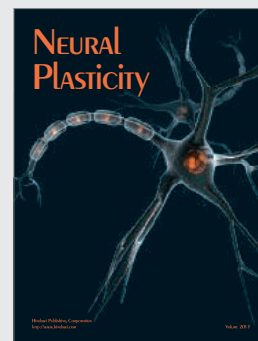
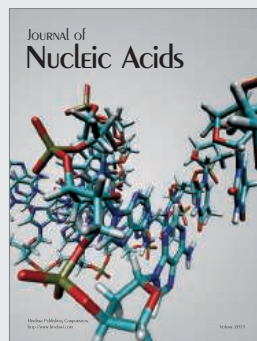
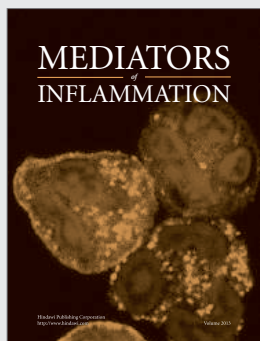
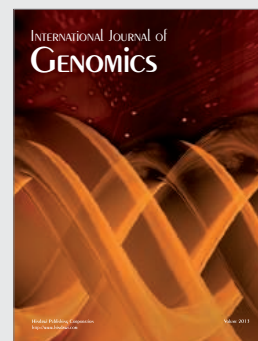
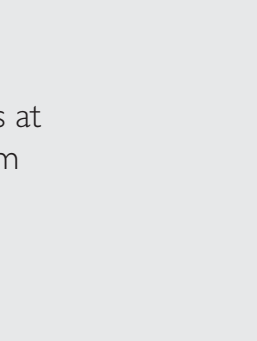
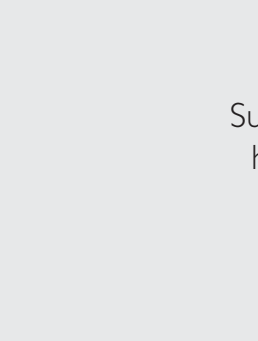
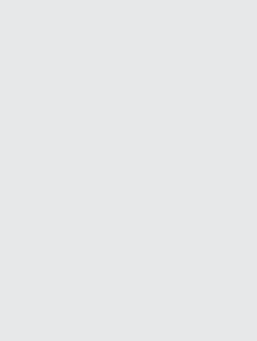
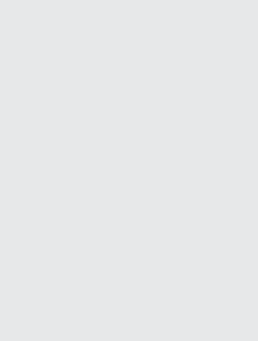
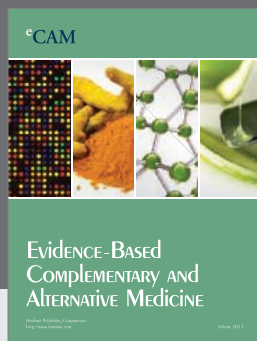
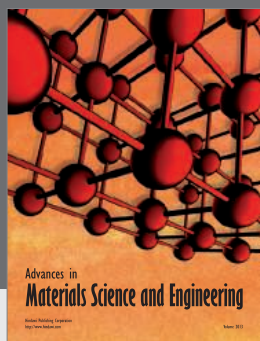
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Supplementary Materials

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Materials and Methods
Figs. S1 to S12
Table S1
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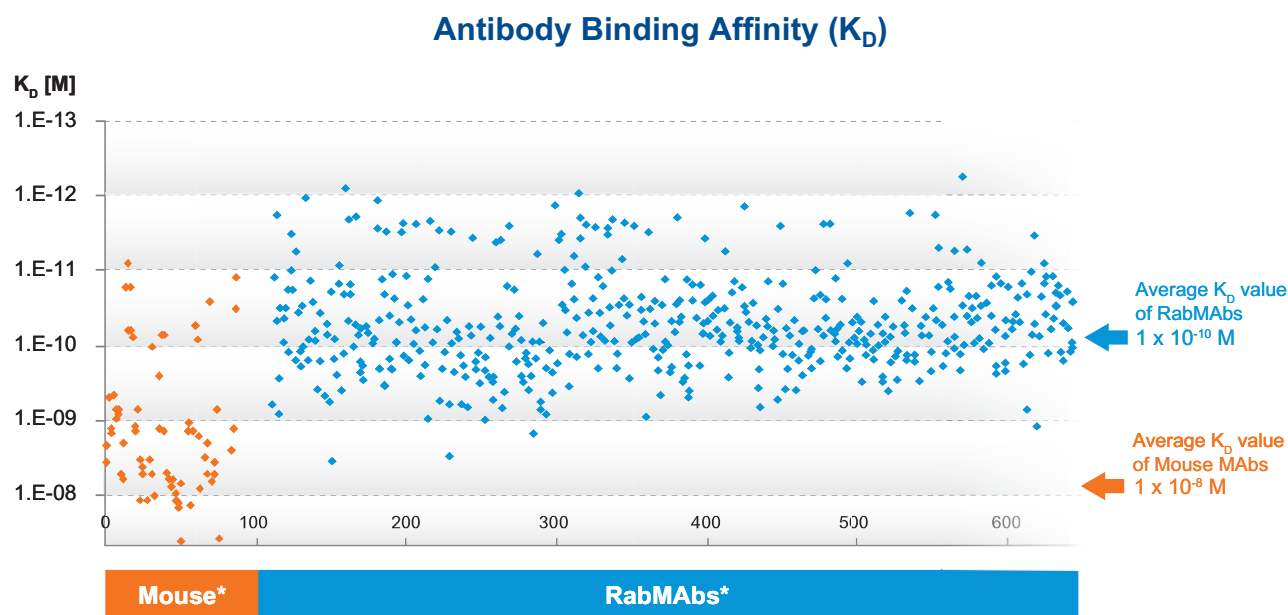
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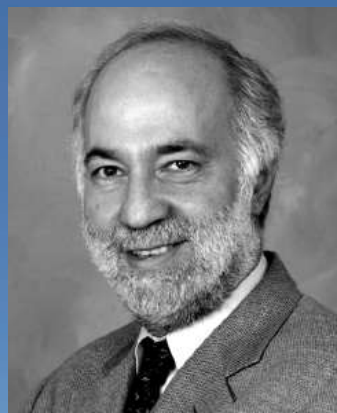
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joint with **Obesity: A Multisystems Perspective (J2)**

Organizers: Roger D. Cone, Barbara Cannon & Lee M. Kaplan
January 12–17, 2014 | Vancouver, British Columbia | Canada
Deadlines: Scholarship/Discounted Abstract – Sep 17; Abstract – Oct 16; Discounted Registration – Nov 14

Emerging Cytokine Networks

Organizers: Daniel J. Cua, Hergen Spits & Federica Sallusto

joint with **Inflammatory Diseases: Recent Advances in Basic and Translational Research and Therapeutic Treatments**

Organizers: Chen Dong, Tadimitsu Kishimoto & Richard A. Flavell
January 17–22, 2014 | Vancouver, British Columbia | Canada
Deadlines: Scholarship/Discounted Abstract – Sep 18; Abstract – Oct 17; Discounted Registration – Nov 18

Pathogenesis of Respiratory Viruses

Organizers: Adolfo García-Sastre & Peter J.M. Openshaw

joint with **Innate Immunity to Viral Infections**

Organizers: Caetano Reis e Sousa, Kate A. Fitzgerald & Charles M. Rice
January 19–24, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Sep 19; Abstract – Oct 22; Discounted Registration – Nov 19

New Frontiers in the Discovery and Treatment of Thrombosis

Organizers: Jane E. Freedman, Bruce Furie & Dietmar Seiffert
January 26–30, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Sep 25; Abstract – Oct 29; Discounted Registration – Nov 25

Mechanisms and Consequences of Invertebrate-Microbe Interactions

Organizers: Bruno Lemaitre, Nicole M. Gerardo & Jason Rasgon
January 26–30, 2014 | Tahoe City, California | USA
Deadlines: Scholarship/Discounted Abstract – Sep 26; Abstract – Oct 30; Discounted Registration – Nov 25

Growth and Wasting in Heart and Skeletal Muscle

Organizers: Elizabeth M. McNally, Nadia A. Rosenthal & Leslie A. Leinwand
January 26–31, 2014 | Santa Fe, New Mexico | USA
Deadlines: Scholarship/Discounted Abstract – Sep 26; Abstract – Oct 30; Discounted Registration – Nov 26

RNA Silencing

Organizers: V. Narry Kim, David P. Bartel & Julius Brennecke
January 31–February 5, 2014 | Seattle, Washington | USA
Deadlines: Scholarship/Discounted Abstract – Sep 30; Abstract – Oct 31; Discounted Registration – Dec 2

The Science of Malaria Eradication*

Organizers: Pedro L. Alonso, Chetan E. Chitnis & Lee Hall
February 2–7, 2014 | Merida, Yucatan | Mexico
Deadlines: Scholarship/Discounted Abstract – Sep 30; Abstract – Nov 4; Discounted Registration – Dec 3

Developmental Pathways and Cancer:

Wnt, Notch and Hedgehog

Organizers: Frederic J. de Sauvage, Mariann Bienz & Jon C. Aster

joint with **Stem Cells and Cancer**

Organizers: Tannishtha Reya, Craig T. Jordan & Philip A. Beachy
February 2–7, 2014 | Banff, Alberta | Canada
Deadlines: Scholarship/Discounted Abstract – Oct 1; Abstract – Nov 5; Discounted Registration – Dec 3

Cancer Epigenetics

Organizers: Sharon Y.R. Dent, Jean-Pierre Issa & Peter A. Jones

joint with **Transcriptional Regulation**

Organizers: Richard A. Young, Robert G. Roeder & Joanna Wysocka
February 4–9, 2014 | Santa Fe, New Mexico | USA
Deadlines: Scholarship/Discounted Abstract – Oct 2; Abstract – Nov 6; Discounted Registration – Dec 4

Plant Signaling: Dynamic Properties

Organizers: Ottoline Leyser, Junko Kiyozuka & Pamela C. Ronald
February 5–10, 2014 | Breckenridge, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Oct 3; Abstract – Nov 7; Discounted Registration – Dec 5

Molecular Cell Biology of Macrophages in Human Diseases

Organizers: Frederic Geissmann, Judith E. Allen & Christopher K. Glass
February 9–14, 2014 | Santa Fe, New Mexico | USA
Deadlines: Scholarship/Discounted Abstract – Oct 8; Abstract – Nov 13; Discounted Registration – Dec 9

Prophylactic and Therapeutic Antibodies

Organizers: Margaret Karow & Neil Stahl

joint with **Biology of B Cell Responses**

Organizers: Hedda Wardemann, Michael G. McHeyzer-Williams
& Michel C. Nussenzweig
February 9–14, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Oct 9; Abstract – Nov 14; Discounted Registration – Dec 10

Omics Meets Cell Biology:

Applications to Human Health and Disease

Organizers: Anne-Claude Gingras, Igor Stagljar & A.J. Marian Walhout
February 18–23, 2014 | Taos, New Mexico | USA
Deadlines: Scholarship/Discounted Abstract – Oct 16; Abstract – Nov 20; Discounted Registration – Dec 16

Mitochondrial Dynamics and Physiology

Organizers: Rodrigue Rossignol & Heidi M. McBride

joint with **The Chemistry and Biology of Cell Death**

Organizers: Guy S. Salvesen, Matthew S. Bogoy & Jennie R. Lill
February 18–23, 2014 | Santa Fe, New Mexico | USA
Deadlines: Scholarship/Discounted Abstract – Oct 17; Abstract – Nov 21; Discounted Registration – Dec 17

The NF-κB System in Health and Disease

Organizers: Alexander Hoffmann & Louis M. Staudt
February 23–28, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Oct 23; Abstract – Nov 22; Discounted Registration – Dec 19



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Long Noncoding RNAs: Marching toward Mechanism

Organizers: Thomas R. Cech, Edith Heard & Ronald R. Breaker
February 27–March 4, 2014 | Santa Fe, New Mexico | USA
Deadlines: Scholarship/Discounted Abstract – Oct 29; Abstract – Dec 4; Discounted Registration – Jan 7

Cilia, Development and Human Disease

Organizers: Elizabeth Petri Henske, Jeremy F. Reiter & Joel Rosenbaum
March 2–7, 2014 | Tahoe City, California | USA
Deadlines: Scholarship/Discounted Abstract – Nov 4; Abstract – Dec 4; Discounted Registration – Jan 7

Parkinson's Disease: Genetics, Mechanisms and Therapeutics

Organizers: Patrick A. Lewis, Thomas Gasser & Marcel P. van der Brug
joint with **Alzheimer's Disease – From Fundamental Insights to Light at the End of the Translational Tunnel**
Organizers: John Q. Trojanowski, Charles F. Albright & Hui Zheng
March 2–7, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Nov 5; Abstract – Dec 5; Discounted Registration – Jan 8

Mobile Genetic Elements and Genome Evolution

Organizers: Nancy L. Craig, Henry L. Levin & Cedric Feschotte
March 9–14, 2014 | Santa Fe, New Mexico | USA
Deadlines: Scholarship/Discounted Abstract – Nov 6; Abstract – Dec 9; Discounted Registration – Jan 8

Inflammation, Infection and Cancer

Organizers: Johanna A. Joyce, Timothy C. Wang & Frances R. Balkwill
joint with **Immune Evolution in Cancer**
Organizers: Suzanne Ostrand-Rosenberg, Olivera J. Finn & Lisa M. Coussens
March 9–14, 2014 | Whistler, British Columbia | Canada
Deadlines: Scholarship/Discounted Abstract – Nov 6; Abstract – Dec 10; Discounted Registration – Jan 9

HIV Vaccines: Adaptive Immunity and Beyond*

Organizers: Nicole Frahm, Susan W. Barnett & Galit Alter
joint with **HIV Pathogenesis – Virus vs. Host***
Organizers: J. Victor Garcia-Martinez, Daria J. Hazuda & Dan R. Littman
March 9–14, 2014 | Banff, Alberta | Canada
Deadlines: Scholarship/Discounted Abstract – Nov 7; Abstract – Dec 11; Discounted Registration – Jan 10

Metabolism and Angiogenesis

Organizers: Peter F. Carmeliet & Michael Simons
joint with **Tumor Metabolism**
Organizers: William G. Kaelin, Jr., Benjamin F. Cravatt III & Peter K. Jackson
March 16–21, 2014 | Whistler, British Columbia | Canada
Deadlines: Scholarship/Discounted Abstract – Nov 18; Abstract – Dec 17; Discounted Registration – Jan 15

Lipid Pathways in Biology and Disease

Organizers: Michael P. Czech, Tobias C. Walther & Morris J. Birnbaum
March 19–24, 2014 | Dublin | Ireland
Deadlines: Scholarship/Discounted Abstract – Nov 19; Abstract – Dec 18; Discounted Registration – Jan 21

Big Data in Biology

Organizers: Lincoln D. Stein, Doreen Ware & Michael Schatz
March 23–25, 2014 | San Francisco, California | USA
Deadlines: Scholarship/Discounted Abstract – Nov 19; Abstract – Dec 18; Discounted Registration – Jan 21

Fibrosis: From Bench to Bedside

Organizers: Jeremy S. Duffield, Steven R. Ledbetter & John P. Iredale
March 23–28, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Nov 20; Abstract – Dec 18; Discounted Registration – Jan 21

Chromatin Mechanisms and Cell Physiology

Organizers: Thomas Jenuwein & Shelley L. Berger
March 23–28 | Oberstdorf | Germany
Deadlines: Scholarship/Discounted Abstract – Nov 20; Abstract – Dec 19; Discounted Registration – Jan 22

Complications of Diabetes

Organizers: Michael A. Brownlee, Matthew D. Breyer & Susan Quaggin
joint with **Innate Immunity, Metabolism and Vascular Injury**
Organizers: Ajay Chawla, Peter Tontonoz & Gwendalyn J. Randolph
March 23–28, 2014 | Whistler, British Columbia | Canada
Deadlines: Scholarship/Discounted Abstract – Nov 21; Abstract – Dec 19; Discounted Registration – Jan 23

The Ins and Outs of Viral Infection: Entry, Assembly, Exit and Spread

Organizers: Karla Kirkegaard, Mavis Agbandje-McKenna & Eric O. Freed
March 30–April 4, 2014 | Breckenridge, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Dec 2; Abstract – Jan 7; Discounted Registration – Jan 29

Novel Therapeutic Approaches to Tuberculosis*

Organizers: Christopher M. Sassetti & Thomas G. Evans
March 30–April 4, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Dec 3; Abstract – Jan 7; Discounted Registration – Jan 29

G Protein-Coupled Receptors: Structural Dynamics and Functional Implications

Organizers: Christopher G. Tate & Fiona H. Marshall
joint with **Frontiers of Structural Biology**
Organizers: Andrew B. Ward & Wayne A. Hendrickson
March 30–April 4, 2014 | Snowbird, Utah | USA
Deadlines: Scholarship/Discounted Abstract – Dec 4; Abstract – Jan 8; Discounted Registration – Jan 30

Exploiting and Understanding Chemical Biotransformations in the Human Microbiome

Organizers: Peter J. Turnbaugh, Curtis Huttenhower & Michael A. Fischbach
April 1–6, 2014 | Big Sky, Montana | USA
Deadlines: Scholarship/Discounted Abstract – Dec 5; Abstract – Jan 9; Discounted Registration – Feb 3

Epigenetic Programming and Inheritance

Organizers: Joseph H. Nadeau, Marisa S. Bartolomei, Peter Gluckman & Wolf Reik
April 6–10, 2014 | Boston, Massachusetts | USA
Deadlines: Scholarship/Discounted Abstract – Dec 5; Abstract – Jan 14; Discounted Registration – Feb 4

Emerging Concepts and Targets in Islet Biology

Organizers: Rohit N. Kulkarni, Raghavendra G. Mirmira & Eckhard Lammert
April 6–11, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Dec 9; Abstract – Jan 14; Discounted Registration – Feb 5

Engineering Cell Fate and Function

Organizers: Darrell J. Irvine, Sangeeta N. Bhatia & Christopher S. Chen
joint with **Stem Cells and Reprogramming**
Organizers: Deepak Srivastava & Shinya Yamanaka
April 6–11, 2014 | Olympic Valley, California | USA
Deadlines: Scholarship/Discounted Abstract – Dec 10; Abstract – Jan 15; Discounted Registration – Feb 6

Adult Neurogenesis

Organizers: Jonas Frisén & Fred H. Gage
May 12–17, 2014 | Stockholm | Sweden
Deadlines: Scholarship/Discounted Abstract – Jan 13; Abstract – Feb 13; Discounted Registration – Mar 12

Autophagy: Fundamentals to Disease

Organizers: Christina H. Eng, Daniel J. Klionsky, Guido Kroemer & Li Yu
May 23–28, 2014 | Austin, Texas | USA
Deadlines: Scholarship/Discounted Abstract – Jan 23; Abstract – Feb 27; Discounted Registration – Mar 24

The Brain: Adaptation and Maladaptation in Chronic Pain

Organizers: Frank Porreca, David Borsook & David W. Dodick
June 15–20, 2014 | Keystone, Colorado | USA
Deadlines: Scholarship/Discounted Abstract – Feb 13; Abstract – Mar 13; Discounted Registration – Apr 15

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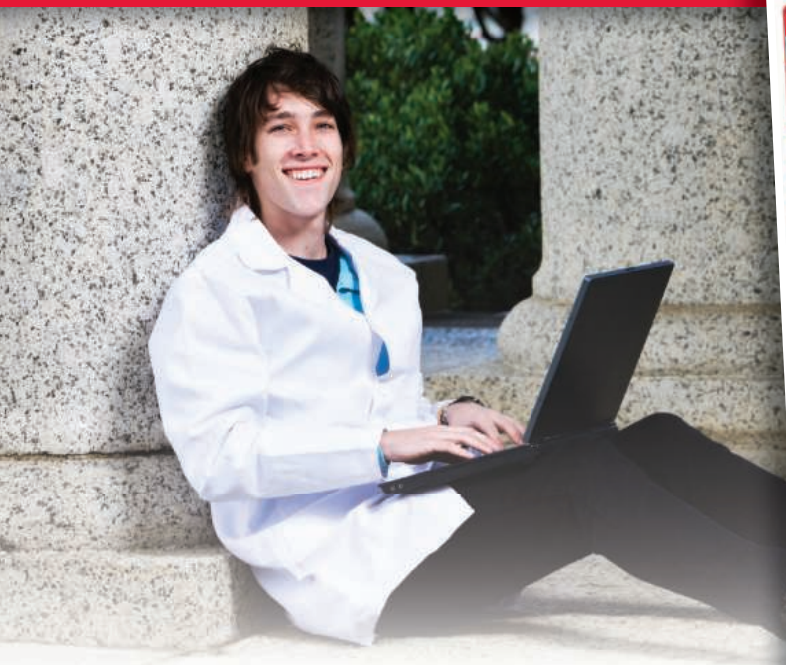
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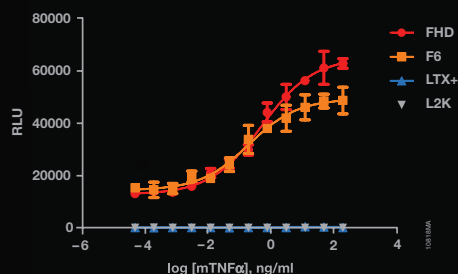
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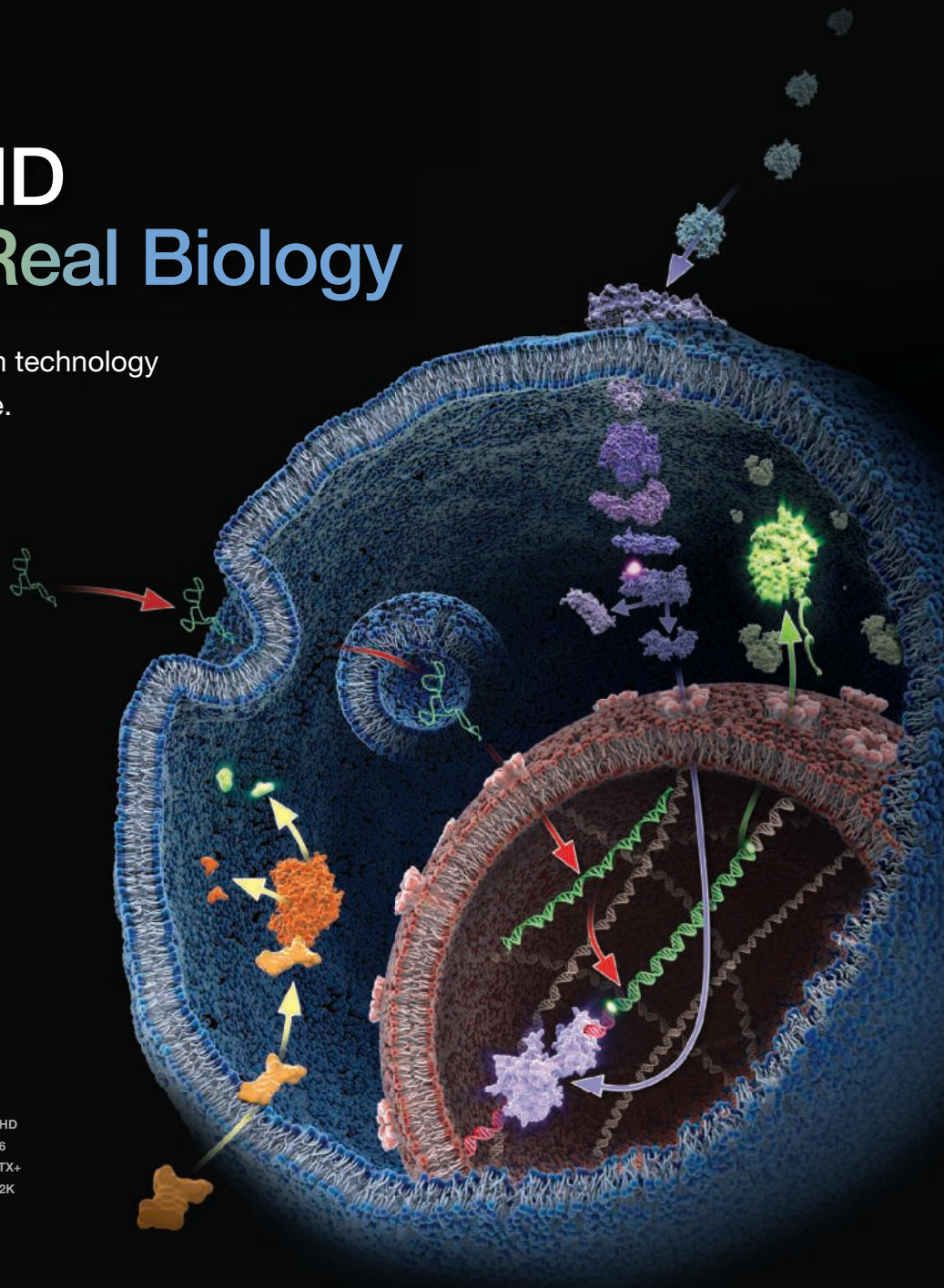
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We are looking for a manager who is a dynamic, motivated individual with the strong organizational and interpersonal skills to coordinate the day-to-day operations and budgetary expenditure of a state of the art multi-user research facility. You will supervise a team of technical staff and liaise with key stakeholders as well as heads of laboratories that use the facility to ensure that University and research OHS&E compliance standards are met. We anticipate the successful applicant will have the vision and skills to implement innovative and exciting new projects utilizing the facility.

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- A Fresh Water Axolotl System. The axolotl facility will be used to maintain a breeding colony of a variety of pedigree axolotls. The facility will house 1.5m tanks for large breeding adults right down to hundreds of small larvae rearing tanks.
- A Marine Research System. This will house a broodstock of Epaullette sharks *Hemiscyllium ocellatum* and an incubation system for the Elephant shark *Callorhynchus milii* embryos.

Aquacore will also include multiple marine tanks of various sizes with the capability to house other tropical and temperate water marine species.

Established through a joint venture between Monash University and the Victorian Government, the **Australian Regenerative Medicine Institute (ARMI)** builds on the University's existing strengths in biomedical research, and supports the critical infrastructure required to deliver the next generation of discoveries in regenerative medicine.

ARMI is located at one of the world's largest regenerative medicine and stem cell research centres, at the Clayton (Victoria, Australia) campus of Monash University. Its scientists are focused on unravelling the basic mechanisms of the regenerative process, enabling doctors to prevent, halt and reverse damage to vital organs due to disease, injury or genetic conditions.

To learn more about us and the work we do please visit www.armi.org. Monash University is an energetic and dynamic university committed to quality education, outstanding research and international engagement. A member of Australia's Group of Eight research intensive universities, it seeks to improve the human condition and is committed to a sustainable future. Monash has six campuses in Victoria, a campus in Malaysia, a campus in South Africa, a centre in Prato, Italy, and numerous international partnerships and cooperative ventures. Monash has over 62,500 equivalent full-time students spread across its Australian and off-shore campuses, and over 7,400 full time equivalent staff. Almost 3,500 of these staff members are academic staff.

Further information regarding this position is available from Professor Peter Currie, Deputy Director, Australian Regenerative Medicine Institute at peter.currie@monash.edu

Interested applicants should send their expression of interest by email to: applications@armi.monash.edu.au

Deadline for applications is 30 September, 2013.

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The Department's research and teaching mission emphasizes developmental biology, stem and progenitor cell biology, tissue and organ formation, tissue repair, regeneration, and aging. These topics are studied at the molecular, cellular, and organismic levels across a number of organ systems by faculty with expertise in the fields of developmental biology, neurobiology, genetics, immunology, hematopoiesis, cancer, cardiovascular development and metabolic disease. A list of HSCRB faculty is available at <http://hscrb.harvard.edu/faculty>.

We seek to hire faculty with a history of innovative research using human, mammalian, or non-mammalian systems. We are particularly interested in applicants who are applying novel tools or experimental and informatics approaches to advance regenerative biology and medicine. Candidates should possess an interest and aptitude in teaching undergraduate, graduate, and/or medical students and will join a dedicated core of scientists and physician-scientists utilizing stem cell and regenerative biology to inform the understanding and treatment of human disease.

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Physician Scientists in the Field of Regenerative Medicine

We are seeking M.D. or M.D./Ph.D. physician-scientists with strong scientific credentials who are interested in clinical translation and who are applying novel tools to advance regenerative medicine – with an emphasis on developing new molecular and cellular therapeutics.

Application Process: Applications, including curriculum vitae, reprints of publications, statement of present and future research plans (1-3 pages), and three letters of recommendation should be addressed to Professors Paola Arlotta and Kiran Musunuru, HSCRB Search Committee, and submitted using the web-form available at <https://academicpositions.harvard.edu/postings/4927>.

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**Robert Wenthold Postdoctoral Research Fellowship in
Communication Sciences**

The Robert Wenthold Fellowship supports NIDCD intramural research training of the most highly promising postdoctoral research fellows seeking to become independent investigators and leaders within the mission areas of NIDCD. The Fellowship includes flexibility in the design of a research training and career development plan with opportunities for multi-disciplinary training, dedicated core facilities, and the breadth and depth of communication sciences expertise within the NIDCD intramural research program (<http://www.nidcd.nih.gov/research/>). NIDCD intramural research includes basic and clinical studies of normal and disordered processes of hearing, balance, taste, voice, speech and language.

The Fellowship is designed to maximize the probability of success for a long-term independent research career in the multi-disciplinary field of communication sciences. Acquisition of new scientific approaches and methodologies is encouraged, with the opportunity for multi disciplinary training and structured co-mentoring. The Fellowship includes an initial two-year commitment of salary, benefits, travel and annual supplies, with the potential for performance based salary increases and extension of the appointment beyond two years.

Eligible individuals will have an MD and/or PhD, or an equivalent doctoral degree, and 0-2 years of prior postdoctoral research experience at the time of initial appointment. Prior postdoctoral experience may be in an NIH intramural or extramural laboratory.

Please submit a curriculum vitae with bibliography, names and contact information of 3 references, up to 3 publication reprints written as first or co-first author, and a brief (<1 page) summary of previous research training and accomplishments, research interests, training and career goals, and a proposal for mentorship and training to: **Ms. Linda de Iberri, Office of the Scientific Director, NIDCD, 5 Research Court, Room 2B28, Rockville, MD 20892 (deiberri@nidcd.nih.gov)**. Applications are due by **October 1, 2013**.

DHHS and NIH are Equal Opportunity Employers and encourage applications from women and minorities.



**Assistant/Associate/Full Professor
School of Molecular Biosciences**

The School of Molecular Biosciences in the College of Veterinary Medicine at Washington State University in Pullman, WA and its new Director Dr. Jonathan Jones, seek to hire outstanding individuals for tenure-track positions at the rank of Assistant, Associate, or Full Professor (<http://www.smb.wsu.edu/>). Successful candidates will have the opportunity to establish their research programs in a state-of-the-art building specifically designed and equipped for biological research. The research interests of applicants should complement the existing focus areas of the School. These include reproductive biology, signal transduction in model organisms, cell adhesion/cytoskeleton and live cell imaging, DNA repair and chromosome biology, microbial genetics and infectious disease. **Duties will include** maintaining a strong, externally funded research program teaching undergraduate and graduate courses and participating in service at the school, college, and/or university level. **Job requirements include** a PhD degree in biosciences or closely related field with a minimum of two years of post-doctoral training in basic science, veterinary medicine, medicine, or a related discipline. Candidates for an assistant professor position must demonstrate potential to establish and maintain an externally funded research program. Applicants for associate and full professor must have an active, established extramurally-funded research program with a record of peer-reviewed publications and national/international recognition. The successful candidate must demonstrate a commitment and relevant ability to successfully advocate diversity and values of diversity.

Applications, including a cover letter, curriculum vitae, and a statement of research interests and teaching philosophy, should be submitted electronically via the www.wsujobs.com/applicants/Central?quickFind=58824. The candidate should also solicit three letters of recommendation to be sent to the same electronic address. Screening of applications will begin early fall. Start date is open and salary will be commensurate with experience. Direct questions to pinter@vetmed.wsu.edu.

Washington State University is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply.



Baylor College of Medicine

**FACULTY POSITIONS
DEPARTMENT OF PHARMACOLOGY
CENTER FOR DRUG DISCOVERY**

The Department of Pharmacology (<http://www.bcm.edu/pharmacology/>) and Center for Drug Discovery (<http://www.bcm.edu/pathimmuno/drugdiscovery/home>) invite applications from outstanding scientists for tenure track positions at the Assistant, Associate or Full Professor rank. A competitive laboratory start up package will be provided to the successful candidates to support the development of an independent, funded research program in drug discovery, chemical biology, protein design and engineering, nanomedicine, pharmacogenomics or other areas broadly relevant to pharmacology. Candidates should have a Ph.D. and/or M.D. degree and postdoctoral experience as well as a strong record of research accomplishments. Baylor College of Medicine is located in the Texas Medical Center and offers a highly interactive environment and a strong infrastructure for research.

Applications should be submitted by **November 1, 2013**. Applicants should submit a statement of research interest, curriculum vitae and the names of three references as a single PDF to pharmacology@bcm.edu.

*Baylor College of Medicine is an Equal Opportunity/
Affirmative Action and Equal Access Employer.*



THE UNIVERSITY OF TEXAS
SOUTHWESTERN MEDICAL CENTER

ASSISTANT PROFESSOR

The Department of Neuroscience at the University of Texas Southwestern Medical Center, under the leadership of Dr. Joseph Takahashi, invites applications at the Assistant Professor level for tenure-track Faculty positions in the broadly defined areas of neurogenetics, genomics, structural biology, electrophysiology and imaging. We seek outstanding scientists addressing molecular and genetic mechanisms underlying behavior, neural circuits and synaptic transmission. Our emphasis is on individuals focused on elucidating molecular, biochemical and biophysical mechanisms of the nervous system. Individuals using advanced functional approaches to study neural circuits are also particularly encouraged to apply. Scientists within the Department of Neuroscience participate in a vibrant, interdisciplinary, interdepartmental, and highly collaborative research community within the University, and enjoy access to state-of-art research cores in imaging, mouse MRI imaging, metabolic phenotyping, behavioral phenotyping, protein chemistry, structural biology, genomics, genetics and transgenic technology.

Applicants should submit a curriculum vitae, two-page summary of research accomplishments and future plans. Applicants should arrange to have 3-5 letters of recommendation sent to the search committee.

Please e-mail application materials to: neuroscienceresearch@utsouthwestern.edu, Neuroscience Search Committee, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9111. The deadline for receipt of applications is **October 1, 2013**.

UT Southwestern Medical Center is an Affirmative Action/Equal Opportunity Employer. Women, minorities, veterans and individuals with disabilities are encouraged to apply.



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, *Science Careers* offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, webinars and downloadable booklets filled with practical advice, a community forum providing answers to career questions, and thousands of job listings in academia, government, and industry, *Science Careers* has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/sciencecareers





INDIANA UNIVERSITY BLOOMINGTON

Faculty Position in Microbiology or Virology

The Microbiology Program (<http://www.bio.indiana.edu/graduate/micro/faculty.php>) at Indiana University invites applications for a tenure-track faculty position at the assistant or associate professor level in **Microbiology or Virology**. We are particularly interested in scientists who combine computational and experimental systems level approaches to study microbial regulatory and metabolic networks or cell biology, although applicants working in other areas of microbiology and virology will also be considered. The successful candidate will be provided with a competitive startup package and salary, and will have access to outstanding research resources. The Indiana University Department of Biology (<http://www.bio.indiana.edu>) and affiliated programs are exceptionally strong in microbiology with more than 40 laboratories working in a variety of prokaryotic and eukaryotic microbes and viruses. Researchers have access to state-of-the-art facilities for genomics and bioinformatics, biological imaging, flow cytometry, protein analysis, analytical chemistry, and crystallography. Applicants must hold a Ph.D. and have relevant postdoctoral experience with a strong record of research accomplishments. Successful junior level candidates will be expected to develop a vigorous externally funded research program. Associate-level candidates are expected to be emerging leaders in their field of research as demonstrated by strong publication and funding records. The successful candidate will participate in teaching at the undergraduate and graduate levels.

Applications received by **October 15, 2013** will be assured of full consideration. Applicants should submit a cover letter, CV, research (past, present, and planned), and a list of three (or more) references using the submissions link at <http://indiana.peopleadmin.com>. Please address inquiries to **Jennifer Tarter** at 812-856-3984.

*Indiana University is an Affirmative Action/Equal Opportunity Employer.
Women and minority candidates are encouraged to apply.*



INDIANA UNIVERSITY BLOOMINGTON

Position in Evolutionary Developmental Biology Department of Biology

The Department of Biology, Program in Evolution, Ecology, and Behavior (EEB) invites applications for an **ASSISTANT PROFESSOR** in the area of **Evolutionary Developmental Biology**. We seek to attract a diverse pool of qualified applicants, with the possibility to hire at the **Associate Professor level in exceptional cases**. We welcome applicants who use a range of approaches to understanding organismal diversification, including the integration of Evolutionary Developmental Biology with Ecology, Genomics, and/or Epigenetics. Applicants must hold a Ph.D. and have relevant postdoctoral experience with a strong record of research accomplishments. For information about the Department of Biology and for links to the campus and the Bloomington community, see **website: <http://www.bio.indiana.edu>**.

Review of applications will begin **October 15, 2013**, and will continue until suitable candidates are identified. Applicants should submit a cover letter, CV, research (past, present, and planned), and teaching statement using the submissions link at <http://indiana.peopleadmin.com>. Applicants should also arrange to have three (or more) letters of recommendation sent to iueeb@indiana.edu. Please address inquiries to **Jennifer Tarter** at 812-856-3984.

Indiana University is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.

UT SOUTHWESTERN MEDICAL CENTER

Faculty Positions in the Center for Autophagy Research

The Center for Autophagy Research at the University of Texas Southwestern (UTSW) Medical Center is a newly created Center designed to foster a collaborative interdisciplinary environment for promoting cutting-edge research in the field of autophagy. The Center is seeking new faculty members at the Assistant Professor (tenure track) level. Faculty will be expected to develop state-of-the-art, innovative independent research programs related to the biochemistry, structural biology, cell biology, genetics, pharmacology, developmental biology or physiological/pathophysiological roles of autophagy. Excellent opportunities exist for collaborations with faculty members in basic science and clinical Departments at UTSW. UTSW is an outstanding scientific environment with established strengths in structural biology, biochemistry, cell biology, microbiology, molecular biology, genetics, and numerous other areas. The positions offer attractive start-up packages and laboratory space. Candidates should have an M.D. and/or a Ph.D. degree with at least 3-4 years of postdoctoral experience and an outstanding publication record.

To apply, submit a C.V., three letters of reference, and a description of research interests to:

**Dr. Beth Levine, Director
Center for Autophagy Research
UT Southwestern Medical Center
5323 Harry Hines Blvd,
Dallas, TX 75390-9113
E-mail: Haley.Harrington@UTSouthwestern.edu**

UT Southwestern is an Equal Opportunity/Affirmative Action Employer.



INDIANA UNIVERSITY BLOOMINGTON

Multiple Faculty Positions in Genomics and Cell Biology

The Genomics, Cell, and Developmental Biology Program at Indiana University invites applications for two tenure-track faculty positions in **Genomics** and in **Cell Biology**. These positions will be filled at the assistant or associate professor level. Candidates should have well-developed research programs that apply cutting-edge approaches to basic questions in eukaryotic biology. Individuals whose research complements/extends our existing strengths are particularly encouraged to apply. Areas of strength in our program include chromatin structure, the cell cycle, chromosome segregation, the cytoskeleton, developmental mechanisms, and signal transduction. Our faculty employ a wide range of organisms, including, but not limited to, *Arabidopsis*, *Drosophila*, mammalian cells, yeast, and protists. All eukaryotic models are welcomed.

The Indiana University Department of Biology (<http://www.bio.indiana.edu/faculty/strengths/index.shtml>) has over 60 research labs housed in 3 adjacent buildings, which contain state-of-the-art facilities for genomics and bioinformatics, microscopy, flow cytometry, protein analysis, and crystallography. Successful applicants will hold a Ph.D. and have relevant postdoctoral experience with a strong record of research accomplishments. Our faculty are expected to establish a vigorous well-funded research program and to participate in undergraduate and graduate education.

Applications received by **October 15, 2013** will be assured of full consideration. Applicants should submit a cover letter, CV, research statement and a list of three references using the submissions link at <http://indiana.peopleadmin.com>. Please address inquiries to **Jennifer Tarter** at 812-856-3984.

*Indiana University is an Affirmative Action/Equal Opportunity Employer.
Women and minority candidates are encouraged to apply.*

Women in Science Booklet

Science and the L'Oréal Foundation present



Read inspiring profiles of women
making a difference in biology.

Free download at
ScienceCareers.org/LOrealWIS

Williams

Tenure Track Faculty Position Biology Department

The Biology Department at Williams College, a premier liberal arts college with a long-standing tradition of excellence in the sciences, invites applications for a tenure-track position at the rank of Assistant Professor, to begin July 2014.

We are seeking a broadly trained **BIOCHEMIST** with solid training in cell biology who will teach in our introductory course in cellular and molecular biology as well as upper level courses in biochemistry and cell biology. The individual will advise undergraduates in research and participate in interdisciplinary programs in biochemistry, molecular biology, and/or bioinformatics. Normally, faculty members teach one course and two laboratory sections (or the equivalent) each semester.

A vibrant research program that is attractive to extramural funding agencies and involving talented undergraduates is expected, and start-up funds and internal funding for research are available. A Ph.D., postdoctoral experience, and a strong research record are required. We anticipate an appointment at the beginning assistant professor level, although more senior appointments are possible under special circumstances. All offers of employment are contingent upon completion of a background check. All applications should be submitted through Interfolio at <https://secure.interfolio.com/apply/22005>. Email and paper applications will not be accepted. Through Interfolio submit: a letter of application addressed to Professor Steve Swoap (department chair), a curriculum vitae, a statement of teaching and research plans, and three current letters of recommendation. Application deadline is **October 18, 2013**.

Williams College is a coeducational liberal arts institution located in the Berkshire Hills of western Massachusetts with easy access to the culturally rich cities of Albany, Boston, and New York City. The College is committed to building and supporting a diverse population of approximately 2,000 students, and to fostering an inclusive faculty, staff and curriculum. Williams has built its reputation on outstanding teaching and scholarship and on the academic excellence of its students. Please visit the Williams College website (<http://www.williams.edu>).

Beyond meeting fully its legal obligations for non-discrimination, Williams College is committed to building a diverse and inclusive community where members from all backgrounds can live, learn, and thrive.

Williams

Tenure Track Faculty Position Biology Department

The Biology Department at Williams College, a premier liberal arts college with a long-standing tradition of excellence in the sciences, invites applications for a tenure-track position at the rank of Assistant Professor, to begin July 2014.

We are seeking a broadly trained **MOLECULAR GENETICIST** who will teach an introductory course in molecular and evolutionary genetics as well as upper level courses in his or her area of specialty. This individual will advise undergraduates in research and participate in interdisciplinary programs in biochemistry, molecular biology, and/or bioinformatics. Normally, faculty members teach one course and two laboratory sections (or the equivalent) each semester.

A vibrant research program that is attractive to extramural funding agencies and involving talented undergraduates is expected, and start-up funds and internal funding for research are available. A Ph.D., postdoctoral experience, and a strong research record are required. We anticipate an appointment at the beginning assistant professor level, although more senior appointments are possible under special circumstances. All offers of employment are contingent upon completion of a background check. All applications should be submitted through Interfolio at <https://secure.interfolio.com/apply/22004>. Email and paper applications will not be accepted. Through Interfolio submit: a letter of application addressed to Professor Steve Swoap (department chair), a curriculum vitae, a statement of teaching and research plans, and three current letters of recommendation. Application deadline is **October 18, 2013**.

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Beyond meeting fully its legal obligations for non-discrimination, Williams College is committed to building a diverse and inclusive community where members from all backgrounds can live, learn, and thrive.

Max Planck Institute for the Physics of Complex Systems

Center for Systems Biology at Dresden

Max Planck Institute of Molecular Cell Biology and Genetics



The Center for Systems Biology (CSB) in Dresden announces the opening of positions in the

ELBE Postdoctoral Program

We seek strong candidates, with backgrounds in Computational Biology, Theoretical Physics, Biophysics, Bioinformatics, Computer Science, and analytically-oriented or quantitative Biology with a strong interest into working in a cross-disciplinary environment and in questions pertaining to Cell and Developmental Biology.

The Center provides a vibrant and collaborative research environment with a strong commitment to the interdisciplinary training and career development of postdoctoral fellows. Successful candidates will benefit from close collaborations with scientists from the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), the Max Planck Institute of the Physics of Complex Systems (MPI-PKS) and the Technische Universität Dresden (TUD).

ELBE Postdocs are awarded on a competitive basis through the postdoctoral program of the Center. To foster collaborations, fellows will usually be affiliated with two Principal Investigators working in different disciplines. For details about the application procedure, please visit our website <http://www.mpg-sysbio.de/jobs.html>.

The Max Planck Society is an equal opportunity employer: handicapped individuals are strongly encouraged to apply. The Center for Systems Biology, the MPI-CBG and the MPI-PKS aim to increase the number of women in scientific positions. Female candidates are therefore particularly welcome.



Faculty Positions Tulane University School of Medicine

The Department of Biochemistry and Molecular Biology is seeking applicants for two positions, the tenure-track Assistant Professor and the Adrouny Endowed Professor as part of a vigorous expansion of the department. The Tulane University School of Medicine (<http://tulane.edu/som/>) offers a highly collaborative scientific environment with many multidisciplinary centers and state-of-the-art core facilities including transgenic, in vivo and in vitro imaging, genomic, and proteomic facilities. Applicants should be established and successful investigators with research interests that complement or strengthen existing research activities in the department (<http://tulane.edu/som/departments/biochemistry/>). Candidates who bring novel approaches and expertise in cancer signaling and metabolism, microRNA, epigenetics, cancer stem cells, medicinal chemistry, tumor immunology, and genetic/disease model systems are especially encouraged to apply. Successful applicants are expected to maintain a strong and independent extramurally funded research program, and to participate in graduate and medical student teaching. The applicants will be provided with a highly competitive salary, significant start-up funds, and excellent laboratory space. Applicants for the tenure-track position must have a Ph.D., M.D. or equivalent degree with several years of postdoctoral experience, and applicants for the endowed position must already have an independent, extramurally funded research program with clear evidence of long-term research excellence. The search committee will begin consideration of applications in September 2013 and continue until April 2014. The positions are expected to be filled by the fall of 2014. Applicants should submit curriculum vitae, a brief summary of past accomplishments, future research plans, and the names and email addresses of three references to: biochem@tulane.edu or by mail to:

The Faculty Search Committee
c/o Gilbert Estrada

Department of Biochemistry and Molecular Biology, #8543
Tulane University School of Medicine
1430 Tulane Avenue
New Orleans, LA 70112

Tulane University is an Affirmative Action/Equal Opportunity Employer and encourages applications from minorities, women, and other qualified persons.

POSITIONS OPEN



TENURE-TRACK FACULTY POSITIONS Department of Biology University of Maryland

The Department of Biology at the University of Maryland, College Park, is seeking to hire several exceptional tenure-track faculty members at any professional rank. Successful candidates will extend, complement, or integrate the Department's existing research strengths in ecology, evolutionary and developmental biology, comparative genomics, sensory neuroscience, and biophysics. Each will be expected to establish a vibrant research program and to be a creative and dedicated teacher at the undergraduate and graduate levels. More information about the Department can be found at **website: <http://www.biology.umd.edu>**. In certain cases, joint appointments with other campus units may be appropriate.

To apply, please visit **website: <https://ejobs.umd.edu/postings/20536>**. Use this site to submit curriculum vitae, a concise statement of current and future research interests, a description of teaching interests, and contact information for three references. Applications received by October 11, 2013 will receive best consideration, but review will continue until all positions are filled.

The University of Maryland is an Equal Opportunity/Affirmative Action Employer. Applications from minorities and women are encouraged.

The Department of Chemistry at the University of Michigan invites applications for an anticipated tenure-track position at any rank in any subdiscipline of chemistry (including analytical, chemical biology, education, inorganic, materials, organic and physical) with a proposed start date of September 1, 2014. This will be a University-year appointment (nine-month academic salary with summer salary supported by research funds). Candidates are expected to develop an internationally recognized program of scholarly research and to excel in teaching at undergraduate and graduate levels.

Detailed information regarding the electronic application process and required materials is available online at **website: <https://www.chem.lsa.umich.edu/chem/facultyrecruit/index.html>**.

Review of applications will begin on October 1, 2013. Information about the Chemistry Department is available on the **website: <http://www.umich.edu/~michchem>**. Questions about the application process should be sent to **e-mail: chemfacrecruit@umich.edu**.

Women and minorities are encouraged to apply. The University of Michigan is supportive of the needs of dual career couples and is an Equal Opportunity/Affirmative Action Employer.

ASSISTANT PROFESSOR-ALL AREAS Princeton University Department of Chemistry

The Department of Chemistry at Princeton University invites applications for a tenure-track assistant professor position in all areas of chemistry. We seek faculty members who will create a climate that embraces excellence and diversity, with a strong commitment to teaching and mentoring that will enhance the work of the department and attract and retain students of all races, nationalities, and genders. We strongly encourage applications from members of all underrepresented groups. Candidates are expected to have completed the Ph.D. in chemistry or a related field at the time of appointment. Applicants should submit a description of research interests, curriculum vitae, a list of publications, and contact information for three references online at **website: <http://jobs.princeton.edu/applicants/Central?quickFind=64207>**. The search committee will begin review of applications on October 17, 2013 and will continue until the position is filled.

Princeton University is an Equal Opportunity Employer and complies with applicable EEO and Affirmative Action regulations.

POSITIONS OPEN

TENURE TRACK/TENURED POSITIONS Center for RNA Biology University of Rochester Medical Center

The Center for RNA Biology (**website: <http://www.urmc.rochester.edu/rna-biology/>**), directed by Lynne E. Maquat, Ph.D., and David H. Mathews, M.D., Ph.D., invites applications for a tenure-track position at the ASSISTANT, ASSOCIATE or FULL PROFESSOR level. We are seeking outstanding candidates holding a Ph.D. and/or an M.D. degree(s) and at least two years of postdoctoral training with research interests in the area of RNA biology. Emphasis is being placed on, but is not limited to, the study of RNA metabolism or the use of RNA as a therapeutic target or tool. Successful applicants are expected to develop independent, externally funded research programs and to contribute toward graduate- and medical-school teaching. The University of Rochester Medical Center and the adjacent College of Arts, Sciences and Engineering are part of a world-class life sciences research campus, providing excellent opportunities for interactions and collaborations, and offer an outstanding research environment with established strengths in RNA Biology. Our Center for RNA Biology has expertise in genome stability, post-transcriptional processing and regulation, structural and functional characterization of molecular interactions, computational biology, and biophysical chemistry. Studies encompass rRNA, pre-mRNA, mRNA, tRNA, lncRNA, and miRNA in humans, model organisms, pathogens, and in vitro.

Applicants should apply online at **website: <http://www.rochester.edu/jobopp>**, job number 176618, and forward their curriculum vitae, descriptions of research accomplishments and future research plans, and letters from three references to **Sharon Kubiak**, Manager of Candidate Administration Services at **e-mail: sharon_kubiak@urmc.rochester.edu**.

The University of Rochester has a strong commitment to principles of diversity and, in that spirit, actively encourages applications from groups underrepresented in higher education.

BIOLOGICAL CHEMISTRY Dartmouth College

The Department of Chemistry at Dartmouth College is seeking an outstanding applicant for an open rank (tenured or tenure-track) faculty position in Biological Chemistry, broadly defined, starting July 2014. We particularly seek candidates who will help lead, initiate, and participate in collaborative research projects both within Chemistry and involving other Dartmouth researchers, including those at Dartmouth's Geisel School of Medicine, Norris Cotton Cancer Center, and Thayer School of Engineering. Teaching responsibilities will include biochemistry and advanced/graduate courses in biological chemistry. The department (**website: <http://www.dartmouth.edu/~chem/>**) is home to 16 tenured and tenure-track faculty with strong Ph.D. and M.S. programs and affiliated with Dartmouth's M.D.-Ph.D. program. Dartmouth College, a member of the Ivy League, is located in Hanover, New Hampshire (on the Vermont border). Dartmouth has a beautiful, historic campus, located in a scenic area on the Connecticut River. Recreational opportunities abound in all four seasons.

Applicants should submit a cover letter, curriculum vitae, a description of research (funding and future plans), and a statement of teaching interests. Senior candidates should provide the names of three references, while tenure-track candidates should arrange for at least three reference letters to be sent. All communications will be treated confidentially. Application materials and reference letters should be submitted to **website: <https://secure.interfolio.com/apply/22014>**; inquiries may be addressed to **e-mail: chemistry@dartmouth.edu**. Applications received by October 1 will receive first consideration. *With an even distribution of male and female students and over a quarter of the undergraduate student population members of minority groups, Dartmouth is committed to diversity and encourages applications from women and minorities.*

POSITIONS OPEN

TWO OPEN FACULTY POSITIONS Boston College Chemistry Department

The Chemistry Department of Boston College invites applications for two tenure-track positions to be effective in the fall of 2014. Applicants will be evaluated based on their potential to establish a prominent and well-funded research program and to excel in teaching at the graduate and undergraduate levels. Successful applicants will join a department of approximately 120 doctoral students, 30 postdoctoral fellows, 200 undergraduate majors, and an internationally recognized faculty.

ASSISTANT PROFESSORS in the (broadly defined) areas of Organic Chemistry and Experimental Physical/Materials Chemistry: requires a Ph.D. in Chemistry or related areas; postdoctoral experience is desirable but not required. The candidate is expected to have published in top refereed journals and demonstrated the ability to perform outstanding independent research.

Interested applicants must submit a cover letter, a graphical executive summary of research plans (one page), curriculum vitae, a summary of research plans (eight pages maximum), a statement of teaching philosophy and arrange to have three letters of reference submitted via the online faculty application at **websites: <https://secure.interfolio.com/apply/21985>** for physical/materials chemistry and **<https://secure.interfolio.com/apply/21965>** for organic chemistry. In the cover letter, the names of three references should be specified.

All application materials must be submitted electronically on or before 15 October 2013.

Boston College, a university of eight schools and colleges, is an Equal Opportunity Employer and supports Affirmative Action.

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From the journal *Science*

